PROTEIN AND AMINO ACID REQUIREMENTS OF FINGERLING CARP (*Cyprinus carpio* L.)

by

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

University of Edinburgh

Department of Forestry and Natural Resources

1983

TO THE MEMORY OF

MY

FATHER

DECLARATION

This thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported within was executed by myself unless otherwise stated.

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ABSTRACT

In terms of dietary concentrations and daily intake, the protein and amino acid requirements of fingerling carp (Cyprinus carpio L.) were determined by using the graded supplementation technique. Assessment criteria were live weight gain, efficiency of food and protein utilisation, and carcass deposition of protein, fat, ash and gross energy.

Optimum protein level was found to be 389 g/kg diet at 20°C. An intake of 280 mg protein/fish/d was adequate for maximum growth rate and protein deposition. The interaction between dietary protein level and lysine concentration was also investigated in factorial arrangement at 20°C. At a protein level of 218 g/kg diet, increased lysine concentrations inhibited growth, whereas at a protein level of 300 g/kg[1] diet growth improved with lysine additions.

The requirement for lysine was examined at 20°C and 25°C. At 20°C the lysine requirement was 14.2-14.5 g/kg diet, which increased to 16.7 g/kg diet at 25°C. When the requirements were viewed in the light of intake, it was shown that the water temperature did not affect lysine requirements of carp, which indicated similar utilisation of lysine at both temperatures. Moreover, it was found that species of fish had no effect on the lysine requirements of carp, tilapia and channel catfish, when these needs are considered as a function of intake. An intake of 4 mg lysine/fish/d promoted similar daily weight gain (0.2 g/fish) in

[1] dry matter

carp, channel catfish and tilapia.

Methionine requirements were studied under a variety of nutritional and environmental conditions. When the DL- isomer of methionine was used as a supplement, the requirements were similar (11.0 g/kg) at 20 and 25°C, and daily intakes of 6.7 and 10.1 mg sulphur amino acids/fish produced maximum growth rate and optimum protein utilisation at the lower and higher temperatures respectively. When the L-form of methionine was used as a supplement, the dietary requirement was estimated at 9.3 g/kg. Carp therefore utilise the L-isomer of methionine more effectively than the DL-form. A daily intake of 4.2 mg sulphur amino acid/fish resulted in an optimum growth rate and maximum utilisation of protein. The replacement value of cystine for methionine was determined to be at least 33% using factorial combinations of both sulphur amino acids. Methionine requirements were 7.8 and 11.0 g/kg diets in the presence of 3.6 and 2.17 g cystine/kg diet respectively. At both dietary methionine levels a daily intake of sulphur amino acids of 3.9 and 4.8 mg/fish was found to be adequate for maximum growth and protein utilisation.

Using the data of all carp experiments involving sulphur amino acids, and those published for channel catfish and tilapia, it was possible to demonstrate broad similarities in methionine requirements of fish. The utilisation of sulphur amino acids by carp appears to be influenced by the dietary proportions of methionine and cystine and the form of

methionine isomer used. In addition, it was found that excessive intake of methionine or cystine retarded the growth of this species of fish.

The requirements for tryptophan, histidine and threonine were assessed at 20°C. The requirement for tryptophan was estimated to be 2.6 g/kg diet. An intake of 1.7 mg tryptophan/fish/d was adequate to support maximum growth and protein utilisation. Histidine and threonine requirements were less than the levels in the basal diets employed: 5.2 and 8.4 g/kg diet respectively.

It was concluded that species, size and age of fish and environmental and nutritional factors may not influence the requirements of fish for protein and amino acids when these requirements are considered in terms of intake.

ACKNOWLEDGEMENTS

Iam most indebted to the University of Mosul for sponsoring my scholarship to study at the University of Edinburgh.

I would like to express sincere gratitude to my supervisors, Dr. D.H. Mills, and Dr. J.P.F. D'Mello for their guidance and invaluable help throughout this work.

I also wish to record my sincere appreciation to the following: Dr. C.B. Cowey, Dr. J. Adron and Dr. J. S. Alabaster for their advice and personal communication in the early stages of this work.

Mr. Keith Easton, Mr. Ken Ryder and Mr. Villis Michels for their help in selecting and providing the desirable size of carp used in this study.

Mr. Jeremy Landless for fish room maintenance and designing some of the electronic circuits.

Mr. Alec Harrower and Mr. David Mackenzie for technical assistance and advice in the workshop.

Miss Pat Phillips for her help and valuable suggestions in the computing and statistical problems.

Mr. Tom Acamovic and the technical staff of the Department of Biochemistry for their help and advice in the chemical analysis.

To the Edinburgh University Library staff for all the help they have given me.

To Miss Mary Anne Richardson for her meticulous care and patience in typing this thesis.

To all my friends and members of the staff of the Department of Forestry and Natural Resources for all their help.

To my brothers, Moyaser and Mowafak Al-salman for their financial help and their continual encouragement.

To my daughter, Suja, for her understanding and patience.

To my wife, Eman, for all her support and encouragement throughout this work.

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List of Abbreviations

α	Alpha
ARC	Agriculture Research Council
BV	Biological value
^{o}C	Celsius
cm	Centimetre
d	Day
DE	Digestible energy
d.f.	Degree of freedom
D-	Isomer of amino acid
DL-	Racemic mixture of amino acid
DM	Dry matter
EAAI	Essential amino acid index
Δ F	Free energy
g	Gram
GE	Gross energy
Δ H	Heat energy
h	Hour
ha	Hectare
kg	Kilogram
kJ	Kilojoule
l	Litre
L-	Isomer of amino acid
m	Metre
M	Mole
mg	Milligram
min	Minute
N	Nitrogen
NAS/NRC	National Academy of Sciences, National Research Council
NPU	Net protein utilisation
DO	Dissolved oxygen
P	Probability
PER	Protein efficiency ratio
SEM	Standard error of the mean
t	T - test
w	Width

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PREFACE

In recent years, more sophisticated methods of fish culture have been developed in order to meet the great demand by humans for fish protein. The success of these fish production techniques is dependent upon the supply of economically practical diets, which should be based on a sound knowledge of the nutrient requirements of each cultured fish species. Such practical diets are now available for the Salmonidae, as studies on the nutritional requirements of these cold water fish have been relatively exhaustive (Halver, 1957a,b; Wood et al, 1957; Halver, 1970; Mertz, 1972; Cowey and Sargent, 1972; NAS/NRC, 1973; Halver, 1976a,b; NAS/NRC, 1977a; Cowey and Sargent, 1979; NAS/NRC, 1981). In contrast, the nutrient requirements of warm water species have received little attention despite their high production potential throughout the world.

Protein is considered to be the most expensive component (NAS/NRC, 1977a) of fish diets since the dietary protein requirements of fish are two to four times higher than those of terrestrial vertebrates (Mertz, 1969). This need could be due to, (i) the high requirements for indispensable amino acids; and/or, (ii) less efficient utilisation of carbohydrates by the fish leading to possible utilisation of some proteins as energy sources.

The requirements of fish for dietary protein and indispensable amino acids have been met through conventional protein sources, of high nutritive value, which are either scarce or very costly

(Hoshia, 1972; Crawford et al, 1974). This difficulty has led nutritionists to search for alternative protein sources which are of high value for fish but cheap and readily available. However, some of these sources have been shown to be of little value for fish due to the limited availability of some of the indispensable amino acids.

As a proportion of the diet, protein requirements of fish are affected by various environmental, physiological and nutritional factors. Since the indispensable amino acids are constituents of protein, the amino acid requirements of fish could be affected by the same factors, when considered in terms of dietary concentrations. The aim of fish culturist is to convert dietary protein efficiently. Therefore, knowledge about the minimum requirements of protein and indispensable amino acids, as a function of dietary concentration or daily intake, is needed to produce maximum growth. This knowledge can help the fish farmer to achieve high production targets.

This study was conducted with a view to providing more information about the protein and amino acid requirements of warm water fish. The carp was selected as the test species since it has a very high growth potential compared to other species of fish being cultured in the warm waters of the world. The utilisation of dietary protein and indispensable amino acids by this fish in relation to various factors constitutes the main theme of this thesis.

I. INTRODUCTION

A. The Test Species - Carp (Cyprinus carpio L.)

1. General characteristics

The carp is a typical warm water fish, belonging to the family Cyprinidae. It is believed to have originated in eastern Asia (McCrimmon, 1968) and has been introduced to many countries in different regions of the world to meet increasing human demand for animal protein consumption. The considerable interest in rearing carp as a productive animal may be related to the growth potential of the species and its ability to tolerate a wide variation of ambient temperatures (5-30°C) and of other environmental factors.

a. Environmental aspects

In general, carp can survive and grow at reduced levels of dissolved oxygen (2-3 mg/l, Hickling, 1962) and can survive extremely high turbidity (up to 20,000 mg of suspended solids/l) with no adverse effects (Stickney, 1979). In addition, carp can adapt themselves to both acid and alkaline waters and may easily tolerate salinities of up to 2,000 mg/l (Hickling, 1962; Bardach et al, 1972).

Carp production is dependent upon temperature and the length of the growing season. The optimum temperature for growth ranges between 23-27°C (Huet, 1972 [23°C]; Harvey, 1978 [25°C]; Stickney,

1979 [27°C]). Since these conditions prevail in the tropical and semi-tropical regions, the growth and maturation of carp is much better in these parts of the world than in temperate zones (Hickling, 1962; Meske, 1968). However, under controlled conditions (indoor rearing) in temperate regions, the growth of carp can be three times greater than in outdoor ponds (Meske, 1968). The success of such a fish cultivation system has encouraged the development of new methods, utilising the warmed cooling water released by power generating stations in Europe (Ghittino, 1972).

b. Systems of cultivation

(i) Extensive

Fish have been cultivated extensively in China for thousands of years (Hickling, 1962; Bardach et al, 1972). This system involved rearing carp at low density in large shallow ponds containing stagnant water. The live food, which the fish find desirable and attractive, is more abundant in this system than in other forms of fish culture.

(ii) Semi-intensive

As a means of satisfying the growing demand for fish protein, the semi-intensive culture system was developed in Europe and has been practised from about the mid 1800s. Fish are reared in smaller ponds and supplied with continuous running water, each pond

being designed to rear fish of a specific age group. Since live food in these ponds is less abundant than in the extensive system, the use of fertilisers (such as phosphorus compounds or cattle manure) and supplementary food (e.g. legumes and vegetables) becomes necessary to increase production (Hickling, 1962; Huet, 1972; Chen, 1976).

(iii) Intensive

In recent years, intensive fish culture (maximum weight of fish in a minimum volume of water) has been developed in several European and tropical countries. Under this system, water and land are utilised more effectively to convert inexpensive dietary protein or carbohydrate sources into high quality fish protein. This system has been successfully applied in Japan (Kawamoto, 1957), which is one of the most important producers of carp (Huet, 1972; Bardach et al, 1972). In certain regions of Japan, carp are raised in small ponds (each 3.3 m x 1.2 m depth) at a very high density (400 kg/pond). Production of carp under such an intensive rearing system is estimated to be 0.6 million kg/ha (Kawamoto, 1957).

Cage culture is a new type of fish cultivation and is being widely used in many countries of the world on production scale. This type of fish culture involves rearing fish at very high densities in net or wooden cages floating in lakes, streams or ponds. Carp could also be reared in floating plastic cages at a very high density (100 kg carp/m^3 in waste water discharged from

power stations at 23-30°C (Ghittino, 1972). The marked increase in production of trout, catfish and salmon in the United States, and carp, eel and yellowtail in Japan is due to the introduction of cage culture (Pillay, 1976).

As is clear from the foregoing discussion, it is possible to intensify carp production by employing cultivation systems similar to those applied to Salmonidae. The fish under such a system of cultivation will be entirely dependent upon the provision of artificial diets. When new, cheap and balanced diets become easily available, the general husbandry technique should shift more towards intensive rearing systems.

c. Digestive physiology

The carp is a stomachless fish, as are many Cyprinidae, but unlike Salmonidae, the existing anterior intestinal swelling appears to function in precisely the same manner as a stomach (Barrington, 1957). Barrington (1957) also reviewed evidence that the length of the intestine is affected by the type of diet and by age. He concluded that carnivores have shorter guts than omnivores, probably due to the nature of the diet, and that older fish have longer guts than younger ones in order to maintain the necessary surface-volume ratio. Loeb (1960) indicated that the forepart of the intestine is large and expansive, but that large meals cannot be eaten. Carp therefore fill the mouth with food which is slowly crushed between the pharyngeal teeth and the basioccipital bone (Loeb, 1960). For the above reasons, digestion

of food by carp could differ from that of other species of fish.

Hickling (1962) reviewed the evidence that various food components are digested in the intestinal tract of carp through the action of endogenous and exogenous enzymes, the latter activated by the former. Protein is digested by trypsin and erepsin and not by pepsin, as in other animals (Ghittino, 1972). Lipid is hydrolysed by lipase and carbohydrates by amylase and maltase. Lichenase is the enzyme responsible for fibre digestion.

More recently, Kawai and Ikeda (1973) studied the activities of the digestive enzymes (protease and carbohydrase) of carp in relation to diet composition. It was found that at constant levels of dietary starch, the intestinal protease activity of young carp increased with higher dietary fish meal concentrations. When maltose, sucrose, lactose or starch were used as carbohydrate sources, maltase and amylase activities in the intestine were high in carp fed diets containing starch or lactose. This may suggest that starch and lactose have the power to stimulate production of digestive enzymes such as intestinal maltase and amylase. However, in comparison with rainbow trout (Salmo gairdneri), carp had a slower increase in digestive enzyme activity. This may have been due to differences in intestinal histology between the species, the amount of diet fed and/or the diet composition which may have affected the rate of synthesis of these enzymes (Barrington, 1957).

2. Food and Diets

a. Natural

The carp is believed to be an omnivorous fish, capable of utilising both animal and vegetable foods (Loeb, 1960, Mackey, 1963, Ghittino, 1972). It has been indicated (McCrimmon, 1968) that newly-hatched carp feed on both zooplankton and phytoplankton and later in life feed chiefly on bottom fauna. In Japan, fish culturists raise water fleas (Daphnia) for very young carp by breeding them in ponds prior to the introduction of newly-hatched fry (Bardach et al, 1972). In Britain, conventional production of young carp relies on the use of the brine shrimp (Artemia) as a first food for fry (Barker and Bryant, 1981). In general, the natural food of carp normally available in ponds contains a high proportion of protein which fulfils all the needs of the young fish (Bardach et al, 1972).

b. Conventional

New, concentrated, high-protein diets have been developed in several countries throughout the world (Ghittino, 1972). These diets are very similar to the dry pellets fed to cold-water fish, and the crude protein (N x 6.25) is derived mostly from fish meal. The level of fish meal recommended for practical carp diets is between 460 (NAS/NRC, 1977) and 480 g/kg (Halver, 1972). The diet formula suggested by NAS/NRC (1977) also contains 50 g of soyabean meal/kg diet. However, fish meal and soyabean are becoming increasingly expensive and the supply is not entirely reliable (Windell et al, 1974; Meyers, 1977; Stickney, 1979; Jackson et al,

1982).

Other very expensive ingredients, e.g. casein and synthetic amino acids, have been used in nutritional research (Stickney, 1979). They are incorporated into purified or semi-purified test diets (Cowey and Sargent, 1972; 1979; Halver, 1970, 1972; NAS/NRC, 1977, 1981) to ascertain the effects of one or more dietary components on the growth and efficiency of food conversion.

As successful large scale cultivation of fish is dependent upon the provision of economic and readily-available protein sources, attempts should be made to replace conventional protein sources in fish diets with unconventional ones which have relatively the same nutritive value.

c. Unconventional

Due to the severe shortage and relatively high cost of fish meal, the potential of various unconventional foods such as poultry (Viola, 1975) and fish processing (Meyers, 1977) by-products, single cell protein (Hoshia, 1972; Windell et al, 1974; Tacon and Ferns, 1976; Butcher, 1978; Meske and Pfeffer, 1978a,b) and leaf protein concentrates (Ogino et al, 1978) has been examined. From among these components, single cell protein seems to show particular promise as a feedstuff for fish. Yeast (Hoshia, 1972) and bacterial petro-protein (Butcher, 1978) have been suggested as protein sources for fish at dietary concentrations of up to 450 g/kg and 500 g/kg respectively, at which level fish meal could be

replaced completely.

Meske (1978) demonstrated that fish meal could be completely removed from the diet of carp when they were fed on a balanced diet containing green algae of the species Scenedesmus obliqus, whey powder, soyabean, amino acid mixture, mineral mixture and trace elements. In contrast, algal protein derived from the species Spirulina maxima was poorly utilised by carp, and was unlikely to be of value as a major component of commercial feeds devised for intensive carp culture (Atack et al, 1979).

The most generally mentioned problems associated with the use of single cell proteins are the toxic residues (Windell et al, 1974) and pathogens present in the material (Tacon and Ferns, 1976). In addition, single cell proteins contain a relatively low content of sulphur-containing amino acids (methionine and cystine).

More attention needs to be focused on the nutritive value of these protein sources, however, before their inclusion in fish diets can be recommended.

B. Evaluation of Dietary Protein

Proteins deficient in one or more of certain amino acids have been found to be incapable of promoting normal growth when fed to animals (Rose, 1938; Rose and Fierke, 1942). Since then, ten amino acids have been identified as "indispensable" or "essential" components which must be supplied in the diet in a balanced

proportion in order to ensure a high growth rate and normal development. Amino acids, other than these ten, have been found to be synthesised by the animal itself and are therefore termed "dispensable" or "non-essential" amino acids. However, it is clear that dispensable amino acids must also be provided in the diet to ensure maximal growth performance and protein deposition (Harper, 1974).

1. Amino acid composition

One of the basic methods of evaluating the protein quality of diets for animals involves estimating the gross amino acid content of food protein. This can be done by microbiological assay or by chemical analysis (McNab, 1979). The amino acid composition of feedstuffs determined by this method, however, gives no precise insight into the actual availability of these amino acids to the animal. This may be due to the adverse effect of processing food protein which usually reduces the availability of certain amino acids, e.g. lysine and methionine (Cowey and Sargent, 1972), and/or causes the destruction of other amino acids, e.g. tryptophan, during the hydrolysis.

It is well known that sulphur amino acids, particularly cystine, cannot be determined accurately by chemical analysis (Blackburn, 1978; Ambler, 1981; Williams, 1981). Cystine is an unstable amino acid during protein or peptide hydrolysis (Blackburn, 1978). In addition, cystine does not actually undergo extensive degradation, but is converted to related derivatives,

e.g. cysteine. Blackburn (1978) also pointed out the difficulties associated with determining methionine by chemical analysis.

For these reasons, determination of protein quality based on chemical analysis does not always agree with the results of biological tests with animals. Nevertheless, the analysis of amino acid composition provides a useful indicator of protein quality and forms the basis of chemical score and essential amino acid index methods.

The chemical score method has been proposed by Mitchell and Block (1946) as a method of evaluating food proteins for growing rats. It relies on comparing the indispensable amino acid content of food proteins under study with that of whole hen's egg protein, considered as a standard of reference. The chemical score may therefore be defined as 100 minus the maximum percentage deficit of an indispensable amino acid in the protein under study, compared to its level in whole egg protein. However, Mitchell and Block pointed out that although the correlation of calculated and experimental values was fairly good in general, there were instances of disagreement.

The essential (indispensable) amino acid index (EAAI) is another measure based upon the gross amino acid composition of food proteins, but unlike the chemical score, it takes into account all the indispensable amino acids rather than the one in greatest deficit. It is defined as the geometric mean of the ratios of indispensable amino acids in a protein compared to those in whole

egg protein (Pike and Brown, 1975). However, both the chemical score and EAAI do not take into account the actual availability of the amino acid in the protein under test. Measurement of the nutritive value of food proteins would therefore be more logical if based on utilisation by the animal for which they are intended.

2. Biological methods

As with other animals, the establishment of a satisfactory procedure for evaluating the nutritive value of dietary proteins is an important prerequisite of fish production. Several techniques have been developed to determine the biological value of food proteins for terrestrial animals (Pike and Brown, 1975). Among these are determination of protein digestibility, protein efficiency ratio (PER), net protein utilisation (NPU), biological value (BV) and efficiency of protein deposition. Due to difficulties associated with determining exact food intake and collecting of faecal materials from the aquatic habitat, the evaluation of food proteins for fish is more difficult and complicated than for other animals.

In feeding trials with mammals, the animals are confined in metabolism cages which separate faecal material and urine by an arrangement of sieves, or else they are fitted with collection bags (McDonald et al, 1981). When representative samples of both food and excreted materials are analysed for protein, the digestibility of protein can be determined. However, digestibility can also be measured in a group of animals by feeding indigestible substances,

e.g. chromic oxide (Cr_2O_3), as an indicator. The ratio between the concentration of the indicator in food and in small samples of the faeces of each animal represents the digestibility.

In the case of fish, three basic techniques have been used to determine the digestibility of food proteins. These methods are: (i) collecting faeces from fish by filtering the aquarium water every 22 h and analysing both food and faeces (Bondi et al, 1957); (ii) using metabolism chambers to collect excretion from gills, as well as urine and faeces (Smith et al, 1980); and (iii) using chromic oxide as an indicator and later sampling faeces, either by killing the fish after predetermined intervals (Plakas and Katayama, 1981) or by filtering the faeces (Choubert et al, 1982).

The first method may be inaccurate since there is the possibility of contamination of the faeces by other nitrogenous compounds, e.g., urine and ammonia. The faeces may also suffer from contamination by nutrients dissolved in water, or through microbial action over the 22 h period. Evidence suggests that most leaching of nutrients occurs during the first hour following defaecation and that a gradual increase in leaching of nutrients continues for up to four hours (Windell et al, 1978). Moreover, this procedure obviously induces stress in the experimental fish because of frequent handling.

Application of the second method also entails stress and restriction of swimming, although it is more accurate than the first . However, utilisation of chromic oxide, incorporated with

quantitative collection of fish faeces (Choubert et al, 1982), represents the most important development in procedures involving determination of protein digestibility. By employing this technique, about 99% chromic oxide recovery could be achieved.

Other biological methods of assessing food proteins in fish nutrition have been reviewed extensively by Cowey and Sargent (1972; 1979). It was concluded that as each of the proposed methods suffered considerable limitation, no generally accepted procedure could be adopted.

PER is defined as the live weight gain per unit weight of protein intake. This measure assumes that all dietary protein intake is used for growth and does not consider the proportion utilised for maintenance. The NPU is the corrected PER for the nitrogen utilised for maintenance. It is determined by the following equation:

$$NPU = \frac{B_f - B_k + I_k}{I_f}$$ (Bender and Miller, 1953)

where B_f and B_k represent the total body protein of the animal on the test and non-protein diets respectively, and I_f and I_k represent the amount of protein consumed by the two groups.

BV is another biological method involving assessment of nitrogen balance in the evaluation of food protein for animals. It

can be calculated by dividing the NPU by the digestibility (Miller and Bender, 1955). However, estimating the protein quality by BV presents difficulties as this method relies upon determination of the N balance of the fish in their aquatic habitat; and ammonia, which is the main end product of protein metabolism in fish (Cowey, 1975), is dissolved in water. For precise measurement by this method, it is necessary to take into account ammonia losses.

Assessing the efficiency of protein deposition or productive protein value entails measuring protein intake and protein retained by the animal during the experimental period. It is defined as g protein deposited/g protein intake. Although similar in several respects to NPU determination, this is,at present, the most useful and most practical method of evaluating food protein for fish (Higuera et al, 1977; Cowey and Sargent, 1979).

Rosenberg (1959) determined the NPU and efficiency of protein deposition of several protein sources in rats. The values obtained for the NPU and efficiency of protein deposition were practically identical. More recently, a study has been conducted (Higuera et al, 1977) on rainbow trout to assess the nutritional value of commercial diets using NPU, BV and efficiency of protein deposition. It was found that the values of efficiency of protein deposition (0.23), NPU (0.3), and BV (0.36) were in close agreement. From this study it was concluded that efficiency of protein deposition was a very suitable method of determining protein utilisation, since it is more accurate and less time-consuming than BV determination, and even easier than

estimation of NPU.

As can be seen, much effort has gone into the development of these mainly experimental methods of assessing the quality of food proteins. Since animals require particular amino acids rather than protein, it is necessary to establish a particular method of protein evaluation on the basis of amino acids that would be available to the animal.

3. Amino acid availability

Amino acid availability may be determined by analysing the animal carcass and excreta (Price et al, 1953) or by faecal and growth analysis (de Muelenaere et al, 1967a,b). Wilson et al (1981) determined the amino acid availability of various feed ingredients for catfish. Their findings represent the most important advance in the evaluation of food protein for fish. Corn, wheat middlings, rice bran, rice mill feed, soyabean meal, peanut meal, cottonseed meal, meat and bone meal and two samples of menhaden fish meal were examined for availability of amino acids after incorporating chromic oxide as an indicator in each ingredient. The apparent amino acid availability was calculated using the formula of Maynard and Loosli (1969). True amino acid availability was calculated employing the formula of Kim (1974) after correcting the apparent amino acid availability data for metabolic faecal amino acids (determined by feeding a protein-free diet). The average true amino acid availability for corn, wheat middlings, rice bran, rice mill feed, cottonseed meal, peanut meal,

soyabean meal, meat and bone meal and two samples of menhaden meal was found to be 79.1, 91.1, 90.1, 76.2, 78.3, 62.4, 84.2, 78.4, 72.5 and 86.1 respectively (see Table 1).

The data on amino acid availability of food ingredients for fish provides valuable information from which the diet manufacturer may formulate a complete practical diet based on the amino acid requirements.

4. Utilisation of free amino acids

As already indicated, due to the severe shortage and relatively high cost of fish meal, several studies have been conducted to evaluate the utilisation of free amino acids as supplements in fish diets. Certain fish species were unable to utilise the amino acid test diet used by Halver (1957b), in which the protein was derived solely from synthetic amino acids. Dupree and Halver (1970) found that channel catfish (Ictalorus punctatus) were unable to grow on diets in which the protein components (casein, gelatin) were partly or wholly replaced by a mixture of amino acids. However, this problem was overcome by adopting a low magnesium and low sulphate salt mixture in place of the mineral salt mixture previously employed.

In another study, channel catfish showed poor growth and low food conversion when menhaden meal was replaced on an isonitrogenous basis by soyabean meal (Andrews and Page, 1974). Addition of methionine, cystine and lysine (the most limiting amino

TABLE - 1

Apparent (A) and True (T) availability of selected amino acids in some ingredients for channel catfish.

Ingredient		Amino acid									
		Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tyrosine	Valine
Corn	A	0.742	0.784	0.573	0.818	0.691	0.617	0.731	0.539	0.687	0.649
	T	0.820	0.903	0.679	0.873	0.965	0.705	0.818	0.698	0.775	0.744
Wheat middlings	A	0.917	0.874	0.818	0.846	0.859	0.767	0.870	0.788	0.830	0.845
	T	0.951	0.945	0.878	0.899	0.963	0.828	0.930	0.891	0.891	0.901
Rice bran	A	0.910	0.704	0.814	0.841	0.813	0.819	0.829	0.773	0.867	0.832
	T	0.942	0.834	0.875	0.905	0.947	0.882	0.895	0.882	0.937	0.892
Rice mill feed	A	0.884	-	0.604	0.665	0.508	0.519	0.621	0.480	0.674	0.720
	T	0.932	-	0.739	0.779	0.743	0.634	0.759	0.672	0.811	0.810
Soyabean meal	A	0.954	0.836	0.776	0.810	0.909	0.804	0.813	0.775	0.787	0.755
	T	0.968	0.879	0.797	0.835	0.941	0.846	0.842	0.822	0.833	0.785
Peanut meal	A	0.966	0.830	0.897	0.919	0.859	0.848	0.932	0.866	0.914	0.896
	T	0.977	0.894	0.933	0.951	0.941	0.912	0.960	0.934	0.945	0.933
Cottonseed meal	A	0.896	0.772	0.689	0.735	0.662	0.725	0.814	0.718	0.692	0.732
	T	0.906	0.816	0.717	0.764	0.712	0.758	0.835	0.767	0.734	0.761
Meat and bone meal	A	0.861	0.748	0.770	0.794	0.816	0.764	0.822	0.699	0.776	0.775
	T	0.879	0.822	0.808	0.824	0.867	0.804	0.854	0.763	0.831	0.808
Menhaden fish meal	A	0.785	0.772	0.656	0.714	0.686	0.630	0.751	0.665	0.750	0.655
	T	0.807	0.836	0.684	0.743	0.722	0.652	0.784	0.710	0.786	0.689

(After Wilson et al, 1981)

acids) to the soyabean-substituted diets did not enhance growth or food conversion. Two reasons could be advanced for this phenomenon: firstly, the differential absorption of free amino acids in comparison with those from the intact protein sources ; and secondly, the low biological availability of other amino acids in the soyabean meal, possibly caused by heat processing during which terminal groups are liable to combine with other compounds (carbohydrate and lipid) or groups (Cowey and Sargent, 1972).

In accordance with the findings of Dupree and Halver (1970) concerning catfish, Aoe et al (1970) found that young carp were unable to grow on diets in which the protein components (casein and gelatin) were partly or wholly replaced by a mixture of synthetic amino acids. The hydrolysates of casein after treatment with trypsin could not replace casein itself, and modification of a binder in the amino acid test diet was also found to be ineffective in maintaining the growth of young carp. Furthermore, modification of the salt mixture, as applied to channel catfish (Dupree and Halver, 1970), failed to bring about any response in carp.

Nose et al (1974) demonstrated that the growth of young carp, fed to satiation six times a day on the amino acid test diet, was only 60% of the growth achieved with fish fed on the casein diet under the same experimental conditions. It was also found that the pH values (<5.0) of the amino acid test diets significantly

depressed the growth rate of carp. However, Nose et al (1974) were able to maintain a satisfactory growth rate on the same test diets after these were neutralised to a pH of 6.5-6.7. The low growth rate of carp offered unneutralised diets could be related to the fact that carp are stomachless fish (Barrington, 1957), and that assimilation of the free amino acids may be enhanced by the strong buffering produced by ingestion of these synthetic amino acids. The failure of young carp to grow on an amino acid test diet in Aoe et al's (1970) experiments could be attributed to the low feeding rate (3% body weight) and to the low feeding frequency (4 times a day). In contrast to this work Plakas and Katayama (1981) have shown that carp absorb amino acids more rapidly from a purified amino acid diet than from an intact protein diet and they suggest that carp have the ability to efficiently utilise the free amino acids derived solely from an amino acid test diet. However, the failure of fish to grow adequately during this experiment casts doubt on the validity of this conclusion.

Jackson and Capper (1982) demonstrated with tilapia (Sarotherodon mossambicus) that supplemented free amino acids could be effectively utilised by the fish. Two diets, each containing a dietary protein level of 400 g/kg, were compared. In the control diet, fish meal, soyabean meal and ground nuts served as protein sources. In the test diet, 50% of the protein was derived from the

same intact protein sources and the remaining 50% was derived from synthetic amino acids (L-form). No significant differences were found between the two diets, which indicates that both diets were of similar nutritional value for tilapia.

Experience with growing pigs has shown that the efficiency of utilisation of supplements of free lysine is affected by the frequency of feeding (Batterham, 1979). In one experiment, pigs were fed a lysine deficient diet either once daily or in six equal portions at intervals of three hours. The diet was supplemented with either 2 or 4 g free lysine/kg diet. It was found that frequency of feeding had no effect on the performance of pigs fed a lysine deficient diet, but that there was a significant ($p<0.001$) interaction between response to free lysine and frequency of feeding. The growth response of pigs fed free lysine under a once daily feeding regime was only 43% of that obtained with frequent feedings. These results indicate that frequent feeding provides a more balanced supply of amino acids at the sites of absorption and metabolism, thereby resulting in more efficient utilisation.

It should be noted that in terms of protein synthesis carp are unable to utilise test diets in which protein is derived solely from synthetic amino acids. The failure of carp to grow on such diets could be due to the excessive acidity created by ingestion of purified amino acids, to differences in gut histology (Barrington, 1957) and/or to the effect of frequency of feeding. However, the feeding regime could play a major role in influencing the efficiency of utilisation of free amino acids in

carp, as was demonstrated in the case of pigs. For the purpose of formulating practical diets for fish, it would therefore be of considerable interest to examine the growth response of fish to free amino acid supplements under different feeding regimes.

5. Nitrogen supplements for fish

The conversion of nitrogen compounds into dispensable amino acids has been studied in pigs (Shelton et al, 1950; Mertz et al, 1952), rats (Lardy and Feldott, 1950), chicks (Stucki and Harper, 1961), and fish (DeLong et al, 1959; Vallet, 1970 in Cowey and Sargent, 1972).

Results of preliminary experiments conducted on weanling pigs have shown that dietary diammonium citrate is an excellent source of nitrogen for protein synthesis (Shelton, et al 1950; Mertz et al, 1952). Similar findings have been reported with rats (Lardy and Feldott, 1950). The nitrogen of diammonium citrate was more efficiently converted to dispensable amino acids than that of urea or glycine. Rogers et al (1970) found that rats were similar to chicks (Stucki and Harper, 1961) and would not achieve their genetically determined growth potential if the dietary protein was derived solely from a mixture of the indispensable amino acids. Additional nitrogen from nitrogenous supplements is therefore necessary for normal growth and development, and the most effective source is a mixture of the dispensable amino acids (Harper, 1974).

Chinook salmon (Oncorhynchus tschawytscha) have been found to

be unable to utilise urea or diammonium citrate effectively. DeLong et al (1959) examined the efficiency of L-arginine.HCl, glycine, urea and diammonium citrate as nitrogen supplement compounds in chinook salmon diets. Six isonitrogenous diets were employed. The basal diet was formulated to contain 200 g protein/kg using casein and gelatine supplemented with a crystalline amino acid mixture in order to give a balance of indispensable amino acids similar to that found in whole egg protein. Diet 1 was designed to contain 400 g of the balanced protein/kg diet. In diets 2-5, the balanced protein was adjusted to 200 g/kg diet and the remaining 200 g protein/kg diet was supplied as the single compound studied: L-arginine.HCl, glycine, urea or diammonium citrate. Growth data indicated that chinook salmon were similar to endothermic animals in their ability to convert arginine and glycine to dispensable amino acids. In contrast to endothermic animals, chinook salmon were found to be unable to convert urea or diammonium citrate to dispensable amino acids (DeLong et al, 1959).

Vallet (1970 in Cowey and Sargent, 1972) has shown with mullet (Mugil sp.) that urea had a sparing effect on dietary protein. At a protein level of 25%, about half of this protein can be replaced by urea for indispensable amino acid synthesis. The ability of mullet to utilise urea could be due to the action of the large numbers of micro-organisms present in the intestine (Vallet, 1970 in Cowey and Sargent, 1972).

More recently, tilapia (tilapia species) were found to utilise

urea as a protein source in experiments conducted in El Salvador (Balarin and Haller, 1982). A dietary concentration of 10 g/kg was successfully included in the experimental diet of this fish.

It should be emphasised that a part of the required protein for chinook salmon cannot be met from urea or diammonium citrate. Whether other cultured fish species are able to utilise urea (as do mullet and tilapia) or other nitrogenous compounds requires further investigation.

C. Protein Requirements

1. Dietary energy and protein utilisation

Fish require free energy (Δ F) for maintenance, growth and biological activities and heat energy (ΔH) for temperature control (Phillips, 1972). Both kinds of energy are components of biological gross energy which represents the total potential dietary energy and which must be available in the diet in the form of protein, carbohydrates and fat.

Gross energy (GE) can be measured after converting the chemical energy of the feed into heat by burning the feedstuff completely to its oxidation products in a bomb calorimeter (McDonald et al, 1981). The GE of feed does not take into account losses in (i) undigested and unabsorbed energy, and (ii) the process of utilisation of the energy itself. Thus, digestible energy (DE) refers to the GE of the ingested feed minus the energy present in faeces voided from

this ingested feed. Metabolisable energy (ME), on the other hand, is the corrected GE for both losses from undigested energy and that energy spent during its utilisation. Net energy (NE) is defined as the energy available to the animal which is actually used for maintenance and production purposes. However, ME is really the most practical and effective means of evaluating the available energy for the different physiological functions of the animal.

Fish utilise feed constituents differently from certain other animals (NAS/NRC, 1977a). Dietary protein and carbohydrates, for instance, are utilised as a primary energy source rather than fats. Thus, the DE of several common feedstuffs, having the same GE value, would be different for fish and pigs. The DE for fish in animal by-products, e.g. poultry feather meal (14.29), fish meal (16.35) and meat meal (14.53kJ/g) is higher than that in the same ingredients for pigs : 11.42, 13.54 and 8.79 respectively as reported by NAS/NRC (1979). In contrast, the DE for pigs in oilseed meal and cereal by-products is higher than that for fish.

The ME values of maize gluten (18.57), fish meal (17.3), soyabean (19.48) and yeast (13.15 kJ/g) obtained by Smith et al (1980) were also higher for fish than those reported by NAS/NRC (1977b) for chicks. However, the ME values of several fish feeds determined by Smith et al (1980) were more accurate and more reliable than those reported by NAS/NRC (1977a), which were originally estimated on the basis of the ME values determined in experiments conducted on poultry.

Energy concentration and its source are among the most important factors affecting energy utilisation. If, for instance, the level of dietary energy provided by fat or carbohydrates is sub-optimal at high dietary protein concentrations, protein can be utilised as an energy source, whereas high energy concentrations at suboptimal dietary protein level may result in undesirable carcass composition of fat. Under the former condition, a proportion of amino acids will be deaminated and the residual carbon will be burnt as energy, while in the latter case, the composition of fish tissue will be affected. In order to avoid both extremes, the sources and levels of energy in the diet have been investigated in relation to protein concentration.

Buhler and Halver (1961) studied the effect of different levels of dextrin, alpha-cellulose and fat on the growth of chinook salmon fingerlings using a casein-gelatin diet containing 360 g protein/kg and supplemented with arginine and methionine. They found that feeding the fish with diets containing different levels of dextrin (0-480 g/kg diet) produced no appreciable differences in growth or health. When the alpha-cellulose level of the diet was varied inversely with dextrin, retardation in fish growth was observed at very high α-cellulose concentrations. This result was interpreted as an effect of increased dietary bulk, but small amounts of α-cellulose in the diet increased the rate of protein synthesis. When dextrin was replaced isoenergetically with corn oil, the growth of chinook salmon was inhibited. In the same study it was also found that galactose retarded the growth of fish and that glucosamine produced the lowest growth when compared with glucose,

maltose, dextrin and potato starch. As can be seen from the results of Buhler and Halver (1961), chinook salmon can readily absorb and utilise relatively high levels of carbohydrate. Whether or not other carnivorous and omnivorous fish utilise such a high level of carbohydrates for protein synthesis needs to be further investigated.

The relationship between dietary protein levels and energy sources was studied in carp and rainbow trout (Ogino et al, 1976). It was found that at low protein levels rainbow trout utilised lipids as a dietary energy source more effectively than carp, whereas carp were capable of utilising carbohydrates (starch and dextrin) more effectively than rainbow trout. This finding suggests a great difference between carp and rainbow trout in utilisation of dietary carbohydrates. The metabolizable energy (ME) values were assumed as follows: 16.6 kJ/g protein, 33.5 kJ/g lipid and 8.4 kJ/g carbohydrate for rainbow trout, and 16.8 kJ/g protein, 33.5 kJ/g lipid and 12.6-20.9 kJ/g carbohydrate for carp.

The interaction of dietary concentrations of protein and energy was examined in channel catfish (Page and Andrews, 1973) using different levels of yellow corn (100, 250 g/kg diet) and fat (6, 12 g/kg diet) at two protein concentrations, 250 and 350 g/kg diet. The DE in these diets was measured by feeding the fish on the experimental diets following inclusion of chromic oxide (1 g/kg diet). The growth rate of channel catfish was found to be affected by the protein and energy concentrations. At an adequate DE level (9.8 kJ/g diet), larger fish (114 g initial weight) had a lower

requirement for protein than smaller (14 g initial weight) fish. This finding was similar to that reported for chicks and pigs, as indicated by Page and Andrews (1973). It was also found that high dietary concentrations of protein and energy resulted in decreased food intake. In addition, the ratio of dietary DE to protein was found to be positively correlated with carcass deposition of fat. In contrast, carcass deposition of protein was not affected by the ratio of dietary DE to protein.

Certain findings were common to most of the studies which examined the relationship between dietary energy and protein concentrations (Cowey and Sargent, 1972). An increase in the ratio of DE and protein resulted in an increased accumulation of lipid in fish tissues. An increase in energy concentrations at a constant dietary protein level led to an improvement in efficiency of food conversion. The ratio of dietary protein and energy was negatively correlated with the PER. Up to 250 g dextrin or starch was found to be an effective energy source. Cowey and Sargent (1979) later emphasised the difficulties in comparing results obtained by different investigators using different values for the ME content.

2. Quantitative protein requirements

The primary aim of fish culture is to transform dietary protein into fish tissue efficiently. In order to achieve this goal, nutritionists have estimated the minimum protein requirements of different cultured fish species which would give maximum growth, maximum protein deposition and maximum economic benefit. However,

little attention has been given to assessing the daily intake of protein required to promote maximum growth rate and carcass deposition of protein in fish.

The protein requirements of most fish species have been investigated by using purified or semi-purified diets as shown in Table 2. These diets were formulated to be deficient in protein and were maintained approximately isoenergetic (assuming that both protein and carbohydrates have the same ME value) by adjusting the dextrin component of the diet. By plotting the growth rate of the fish against the protein level used, growth responses could be drawn (as will be shown later in Figs. 1 and 2). From this, the protein requirement could be fixed at the point of intersection between the plateau line and the linear portion of the response curve (Cowey and Sargent, 1972).

DeLong and his co-workers (1958) were the first to estimate the protein requirement of chinook salmon fingerlings. Fish were fed on partially defined diets consisting of gelatin, casein and a mixture of crystalline amino acids (adjusted to approximate that of whole egg protein). The test diets were formulated to contain different levels of protein (50-650 g/kg DM) by substituting dextrin for protein on a weight basis. The results of this experiment showed that chinook salmon fingerlings required 400-550 g protein/kg diet.

Many investigators employed the above dietary procedure (DeLong et al, 1958) to determine the protein requirements of other species

TABLE - 2

Estimated dietary protein requirements (g/kg) of eight species of fish.

Fish species	Stage of life	Water temperature (^{o}C)	Source of protein used	Requirement
Tilapia[1]	Fingerling	25	Casein	350
Chinook salmon[2]	Fingerling	8	Casein, gelatin and amino acid mix.	400
Chinook salmon[2]	Fingerling	14	casein, gelatin and amino acid mix.	550
Plaice[3]	<1 (year)	15	Freeze dried cod muscle	500
Eel[4]	Elver	25	Casein and gelatin	450
Channel catfish[5]	Fingerling	15	Casein, wheat gluten and soyabean	350
Channel catfish[5]	Fingerling	18	Casein, wheat gluten and soyabean	400
Channel catfish[6]	> 1 (Year)	25-28	Casein	253
Milkfish[7]	Fry	25-28	Casein	400
Grass carp[8]	Fry	22-23	Casein	423
Carp[9]	Fry	23	Amino acid mix.	380
Rainbow trout[10]	Fingerling	9-12	Casein and gelatin	400-450
Rainbow trout[11]	Fingerling	16-27	Fish meal	400

[1] Mazid et al (1979)
[2] DeLong et al (1958)
[3] Cowey et al (1972)
[4] Nose and Arai (1973)
[5] Dupree and Sneed (1966)
[6] Nail (1962)
[7] Lim et al (1979)
[8] Dabrowski (1977)
[9] Ogino and Saito (1970)
[10] Zeitoun et al (1973)
[11] Satia (1974)

of fish. The results of these studies were similar in qualitative terms to those obtained by DeLong and his colleagues. A linear increase in growth rate was observed as the dietary protein increased up to a certain level (Dupree and Sneed, 1966; Ogino and Saito, 1970; Cowey et al, 1972; Nose and Arai, 1973; Dabrowski, 1977; Lim et al, 1979; Mazid et al 1979).

Dupree and Sneed (1966) found with channel catfish fed on purified diets, wheat gluten or soyabean proteins, that weight gains were linear with the protein content of diets up to approximately 350 g/kg DM. A greater amount of protein reduced the weight gain.

Ogino and Saito (1970), working with carp, used diets containing casein as a source of protein. Highest body weight was obtained when the diet contained 550 g protein/kg. It was suggested that a dietary protein level of 380 g/kg could be optimal for carp as protein deposition did not increase at higher dietary protein levels.

Cowey et al (1972) conducted experiments with plaice to determine the growth responses to six diets containing 20-70 g dietary protein in increments of 10 g/kg using casein at various levels. A linear growth rate resulted as the dietary protein increased up to a level of 700 g/kg with no evidence of its levelling off. It was estimated that young plaice (Pleuronectes platessa) required a dietary protein level of 500 g/kg for optimum growth performance.

Nose and Arai (1973) determined the protein requirements of eel (Anguilla japonica) using a range of dietary protein levels from 0 to 523 g/kg. A linear relationship was found between the dietary protein level, up to 445 g/kg, and carcass deposition of protein. There was no obvious increase in carcass deposition of protein at higher protein levels. It was therefore concluded that the eel required approximately 450 g protein/kg diet for normal growth.

Similarly, grass carp (Ctenopharyngodon idellus) exhibited a linear relationship between the dietary protein level and carcass deposition of protein up to dietary protein levels of 410-430 g/kg (Dabrowski, 1977). No further increase was observed in carcass deposition of protein in fish fed diets containing higher levels.

Mazid et al (1979) demonstrated that tilapia fingerlings required 350 g protein/kg diet when casein was used as the sole source of protein. For optimum growth, a 300 g protein/kg diet was required for maximum bodily protein deposition.

Lim et al (1979) fed milk fish (Chanos chanos) fry five semi-purified diets containing 200, 300, 400, 500 and 600 g protein/kg. A linear relationship was found between the growth rate of fish and dietary protein levels of up to 400 g/kg. A dietary protein level of 400 g/kg was therefore considered as optimum for the growth rate of milk fish.

From the evidence presented in this section, it becomes clear that the protein requirements of fish differ from one species to

another when considered in terms of dietary concentrations, and expressing the requirements in this manner largely accounts for these differences. However, an interesting picture could emerge if the protein requirements of fish were considered in terms of daily intake, as such a criterion would help to distinguish those factors, such as species, which exert their effects through changes in voluntary food intake (see next Section).

3. Factors affecting the protein requirements

Several factors have been implicated in influencing protein requirements of fish. These include genetic factors (DeLong et al, 1958; Mertz, 1969; NAS/NRC, 1977a), environmental factors (DeLong et al, 1958; Halver, 1976a,b), age or body size (Satia, 1974; Halver, 1976a; NAS/NRC, 1977a; Balarin and Haller, 1982), and several other nutritional factors such as source (Ogino et al, 1976) and level (Parther and Lovell, 1973 in NAS/NRC, 1977a) of dietary energy ratio and dietary amino acid imbalance.

a. Genetic factors

The protein requirements of fish (400-550 g/kg diet) are 2-4 times higher than those of birds and mammals (DeLong et al, 1958; Mertz, 1969; NAS/NRC, 1977a). The plasma amino acid levels in actively feeding chinook salmon have been compared with those of actively feeding pigs (Mertz, 1969). The levels of the individual indispensable amino acids were three to six times higher in salmon than in pigs. This result may indicate the ability of fish species

to tolerate high protein or plasma amino acid levels which permit a faster rate of protein synthesis in the body. The higher plasma amino acids in fish could be attributed to the reduced levels of those enzymes which are responsible for the deamination of amino acids (Mertz, 1969). Another explanation for the higher protein requirements of fish could be related to their greater need for indispensable amino acids as compared with other species of animals (see Section I-E-2 and Table 14).

b. Environmental factors

Since fish are ectothermic animals, environmental temperature is one of the factors which greatly affects their protein requirements when expressed as dietary concentrations. The response of chinook salmon to graded dietary protein concentrations (Fig. 1) has been examined at two water temperatures, 8 and 14°C, by DeLong and his colleagues in 1958. The requirements of chinook salmon for dietary protein were found to be 400 and 550 g/kg respectively at the two temperatures tested. Similarly, Dupree and Sneed (1966) found that the optimum levels of dietary protein for channel catfish were 350 g/kg diet at a water temperature of 15°C and 400 g/kg at 18°C. Halver (1976a,b) reported that the dietary protein requirements of rainbow trout and chinook salmon were similar when raised at the same temperature.

When requirements are expressed as dietary concentrations, it is clear that there are differences in the protein requirements of fish at different water temperatures. Such data, however, does not

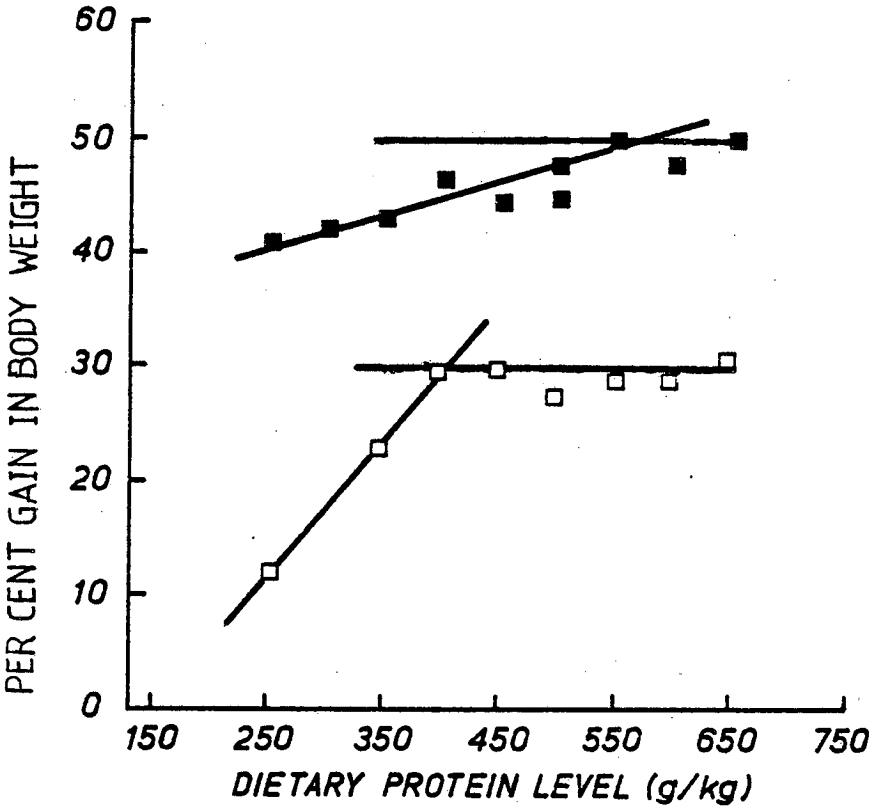


Fig. 1 Protein requirement of chinook salmon at two water temperatures, 8 (□) and 14°C (■). (After DeLong et al, 1958)

provide information about the utilisation of protein by fish at different water temperatures. In other words, fish may need more dietary protein at higher temperatures than those reared at lower temperatures and growing at similar rates, either because the fish at lower temperatures utilise dietary protein less efficiently or because they consume less food. These questions cannot be answered without recourse to reliable data on growth and food intake of fish reared at different environmental temperatures.

Zeiton et al (1973) have shown that the protein requirement of rainbow trout fingerlings was affected by the osmotic pressure of the water in which they were reared. Seven concentrations of dietary protein ranging from 300 to 600 g/kg diet were fed at two salinity levels of 10 and 20 mg/l. The protein requirements were found to be 400 and 450 g/kg diet at the two salinity levels. From these results it is clear that the protein requirement, as g/kg diet, varies with water salinity in the same manner observed with water temperature. Whether these environmental factors affect protein utilisation by fish calls for further investigations.

c. Age and size

As in other terrestrial animals (NAS/NRC, 1977b; McDonald et al, 1981), the protein requirements of fish decrease as the animal becomes older and stores relatively less protein and more fat in each unit of gain. Satia (1974) demonstrated with rainbow trout that the dietary protein requirements of older fish were lower than those of young fish. It was also indicated that the requirement of

trout and salmon for dietary protein decreased as the fish increased in size (Halver, 1976a,b; NAS/NRC 1977a). Channel catfish fingerlings require about 400 g/kg diet (Dupree and Sneed, 1966) which decreases to 253 g/kg diet at the age of approximately one year or more (Nail, 1962). Similar findings were also reported with channel catfish by Page and Andrews (1973).

In recent years, several studies have been carried out by various investigators (see Balarin and Haller, 1982) to estimate the protein requirement of tilapia for maximum growth in various size groups. The requirement of young tilapia of the size group below 1 g was a dietary protein level of 350-500 g/kg. This concentration of dietary protein decreased to 200-250 g/kg as the size of the fish increased up to 25 g or more (Balarin and Haller, 1982). From the data presented in Table 2 and from the foregoing discussion, it is clear that the requirements of fish vary with age and size, and that these requirements should also be recognised as a function of intake.

As mentioned above and shown in Table 2, most studies on the quantitative protein requirements of fish are carried out with small fish of fry to fingerling size. It should be recognised that at such a stage of life fish grow at a rapid rate, and that their dietary protein requirements may not remain constant. It therefore becomes necessary to define the quantitative protein requirements of adult fish.

d. Nutritional factors

The theory of "extra energetic effect" of fat has been proposed for chicks by Renner (1964). An experiment was conducted to investigate the role which protein plays in enabling chicks to utilise "carbohydrate-free" diets in which non-protein energy is supplied by fat. Neither growth nor protein retention decreased when fat was substituted isoenergetically for glucose in diets containing 55.3, 64.5, 73.7, 82.9 and 92.1 kJ/g protein. These results indicated that energy requirements of the chick could be met equally from fat or carbohydrate without diverting amino acids from protein to carbohydrate synthesis.

Studies with channel catfish (Tiemeier et al, 1965; Prather and Lovell, 1973 in NAS/NRC, 1977a) and rainbow trout (Ogino et al, 1976) have shown that the protein requirements of these fish are affected by the source and level of energy in the diets.

Ogino et al (1976) fed rainbow trout on six test diets containing dietary lipid of (1) 270, (2) 180, (3) 100, (4) 20, (5) 30 and (6) 30 g/kg respectively. The dietary dextrin levels were adjusted to 150 g/kg in diets 1-4, and 550 and 400 g/kg in diets 5 and 6 respectively. The results of this experiment showed that the protein level required to achieve maximum growth rate was approximately 300 to 350 g/kg diet when the protein and energy levels in the diets were adjusted with cellulose and lipid. But this estimate shifted to about 400 g/kg diet when carbohydrate formed the major energy source (Fig. 2).

However, the data of Ogino et al (1976) may be viewed in a

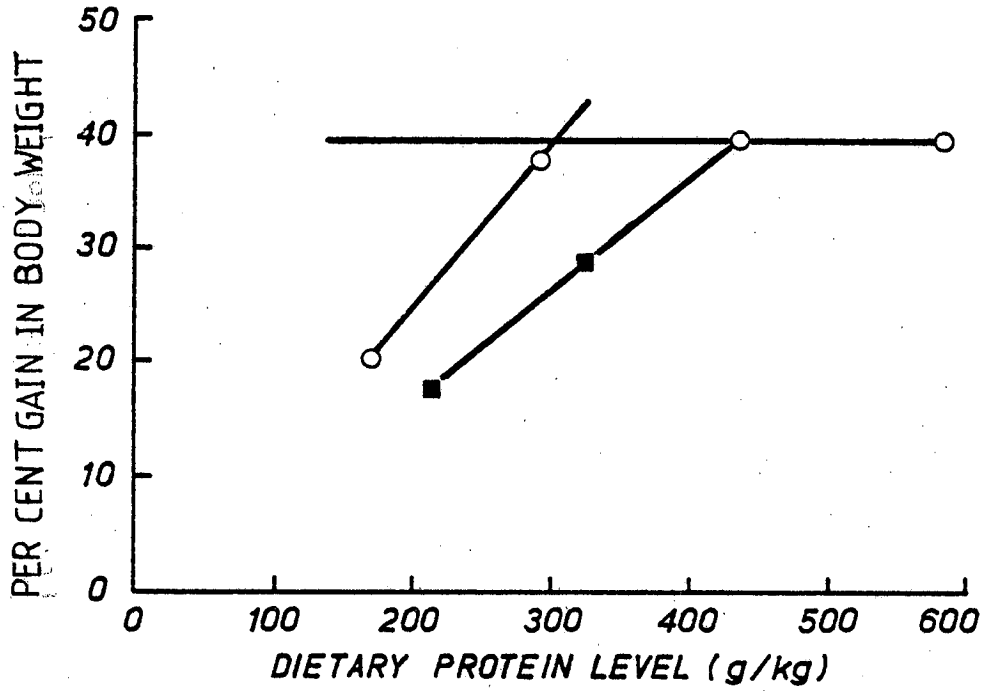


Fig. 2 The relationship between dietary protein levels and growth rate of rainbow trout when lipid (○) or carbohydrate (■) was used as main dietary energy source. (After Ogino et al, 1976)

different light. It may be proposed that food intake of trout is affected by energy source. Furthermore, protein utilisation ought to be measured and expressed in terms of retention or deposition in carcass. If the results of Ogino et al (1976) are now considered in terms of protein deposition and daily protein intake, a single response curve (Fig. 3) is obtained, which demonstrates clearly that protein utilisation is unaffected by the source of dietary energy. It appears that fish are similar to chicks (Renner, 1964; ARC, 1975) in this respect.

Cowey and Sargent (1972), NAS/NRC (1977a) indicated that the protein requirements of fish were also found to be directly affected by the indispensable amino acid pattern in the diet (see I-E-3-d).

From the foregoing discussion, it is clear that the protein requirements, as dietary concentrations, of fish are dependent upon several factors. It is of considerable interest, however, to establish whether protein utilisation in fish is influenced by the same factors when requirements are considered in terms of daily intake. More research therefore needs to be conducted to determine protein requirements of fish as a function of intake under the relevant conditions.

D. Amino Acid Requirements: Qualitative Aspects

1. Identification of indispensable amino acids

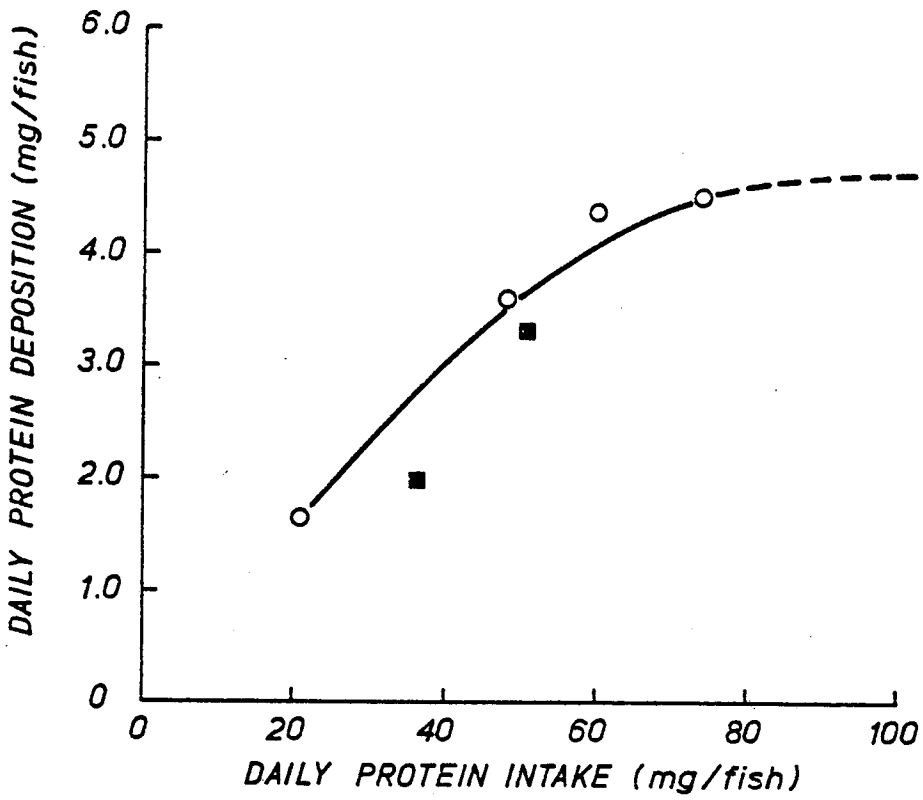


Fig. 3 The relationship between daily protein intake (mg/fish) and daily protein deposition (mg/fish) of rainbow trout when lipid (O) or carbohydrate (■) was used as main dietary energy source. Data calculated from results of Ogino et al (1976)

Early studies with young albino rats have shown the importance of certain amino acids for normal growth and development (Rose, 1938; Rose and Fierke, 1942). These amino acids (Table 3) have been identified as "indispensable" amino acids since animals are unable to manufacture their carbon skeletons or their corresponding keto acids.

On the other hand, the amino acids which the animal can synthesise within its own tissues are termed "dispensable" amino acids.

Rose and his colleagues (1938) formulated a diet in which all the protein was supplied by crystalline amino acids of high purity. On the basis of omission tests, they were able to classify the indispensable amino acids for rats. But identification of the indispensable amino acids for fish represents a major problem since their aquatic habitat may affect the consistency of the experimental diets.

Halver et al (1957b) designed, and used for the first time, a successful amino acid test diet based on the same amino acid pattern of a casein-gelatin diet (Halver, 1957a) used to determine the vitamin requirements of chinook salmon. A test diet, similar in amino acid composition to that found in yolk-sac fry and fingerling salmon protein, failed to promote acceptable growth when compared with the casein-gelatin diet which maintained chinook salmon fingerlings at a reasonable growth rate (0.14 g/week) for at least fourteen weeks. Amino acid deficient diets were formulated

TABLE - 3

The nutritional classification of amino acids.

Indispensable	Dispensable
Arginine	Cystine
Histidine	Tyrosine
Isoleucine	Glycine
Leucine	Serine
Lysine	Proline
Methionine	Alanine
Phenylalanine	Aspartic acid
Threonine	Glutamic acid
Tryptophan	
Valine	

by omitting one amino acid from the basal diet (complete diet) and replacing it with an equal weight of α-cellulose flour. Growth on these diets was compared with that obtained with the basal diet. Results showed that chinook salmon required the same ten indispensable amino acids as those needed by rats and other animals. A dietary source of tyrosine, glycine, alanine, aspartic acid, glutamic acid, cystine or proline therefore appeared dispensable for the growth of this species of fish. The same procedure was followed by many researchers to classify the indispensable amino acids for channel catfish (Dupree and Halver and, 1970), carp (Aoe et al, 1970) and tilapia (Mazid et al, 1978).

Dupree and Halver (1970) found by using the same amino acid test diet fed by Halver (1957b) to chinook salmon, that poor growth occurred in channel catfish fingerlings. However, the adoption of a salt mixture with low Mg and low SO_4 was helpful in promoting growth in channel catfish.

Nose et al (1974) used amino acid test diets to classify the indispensable amino acids for carp after neutralising the diet with a necessary amount of 6M NaOH. They noticed a very low feed efficiency (15-20%) and inferior growth in young carp on the amino acid test diet compared to those on the casein diet. Despite the poor food utilisation, the authors concluded that young carp required the same ten, indispensable amino acids as chinook salmon and channel catfish for normal growth and development.

Cowey et al (1970) used more sophisticated methods of

identifying the indispensable amino acids for plaice and sole (Solea solea). Fish were given intrahaemocoelic injections of uniformly ^{14}C-labelled glucose. After treatment, the level of radioactivity in the expired carbon dioxide and other labelled metabolites was monitored for six days in order to estimate the total recovery of radioactivity. The fish were killed, the tissue protein isolated and hydrolysed, the constituent amino acids resolved and purified, and then examined for radioactivity. Those amino acids which contained radioactive material could clearly be synthesised by the fish itself from ordinary available materials and were considered to be dispensable dietary constituents. On the other hand, those amino acids which did not contain radioactivity could not be synthesised by the fish and were classified as indispensable amino acids.

The ability of many animals to synthesise cystine and tyrosine from their precursors, methionine and phenylalanine respectively, is well recognised as long as the diets contain sufficient amounts of the appropriate precursors. Both cystine and tyrosine are therefore classified as dispensable amino acids for those animals. However, on the basis of the technique used by Cowey et al (1970), the absence of label in the carbon atoms of cystine and tyrosine implies indispensability of these amino acids. The real reason for the absence of label in cystine and tyrosine is that their precursors are not synthesised in the first place. The technique of Cowey et al (1970) should be viewed with caution. Thus dispensability of cystine and tyrosine can only be established by the use of synthetic diets.

The pattern of indispensable amino acids that is required by fish which have so far been studied is similar to that of endothermic animals. These amino acids must, therefore, be included in fish diets as necessary for growth and development. However, fish are unable to synthesise arginine in their own tissue and are therefore similar to chicks in this aspect.

2. Effect of indispensable amino acid deficiency

A deficiency in essential nutrients such as amino acids would be expected to cause a deterioration in the normal growth, development and health of an animal. A survey of the literature on this subject revealed that in-depth studies on the effects of amino acid deficiency in fish were almost non existent. Most of the available information reported in this area has been obtained during classification of the indispensable amino acid requirements of some fish species.

Studies with rats (Rose 1938; Rose and Eppstein, 1939), chicks (Almquist and Grau, 1944; Ousterhout, 1960) and pigs (Mertz et al, 1952) have shown that the dietary deprivation of an indispensable amino acid can affect the general health of animals. The biochemical changes induced by such deficiencies and their effect on growth, food intake and body composition were reviewed extensively by D'Mello and Lewis (1978) with particular attention to rats. Growth retardation and biochemical changes in the tissues of the animal, leading in some instances to death, was found to be dependent upon the missing indispensable amino acids. Ousterhout

(1960) indicated, for instance, that early deaths in chicks occurred when they were fed on diets lacking either isoleucine or valine. This observation was attributed to a severe amino acid imbalance resulting in body tissues which limited the synthesis of necessary protein. The chicks fed diets lacking in lysine or histidine lost less weight, lived longer and were stronger and more vigorous than chicks fed diets deficient in the other indispensable amino acids. These results led to the investigation of a body store of these amino acids, e.g. haemoglobin (Ousterhout, 1960).

Sockeye salmon (Oncorhynchus nerka) (Halver and Shanks, 1960) and eel (Nose, 1969; Arai et al 1972) have been reported to show retarded appetite shortly after being fed an amino acid test diet deficient in an indispensable amino acid. Dupree and Halver (1970) recorded daily observations on channel catfish fed on complete and amino acid deficient diets. From these observations they established a close association between fish activity and weight loss. Fish fed the control diet and diets devoid of the dispensable amino acids were very active and searched continuously for food. In contrast, fish fed on diets lacking the indispensable amino acids soon became listless, remained on the bottom of the aquarium and were very lethargic when food was offered or when disturbed by laboratory personnel.

In the same experiment, channel catfish fed on diets deficient in tyrosine, glycine, alanine, aspartic acid, glutamic acid, cystine or proline showed similar or slightly lower weight gains than those fed the complete amino acid diet. The group fed on

diets deficient in arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan or valine lost 2-4% in weight. The fish fed on the deficient diets were divided into sub-groups. The sub-group fed the complete amino acid diet gained 36-51% in weight, whereas the second fed continuously on an amino acid-deficient diet lost a further 3-6% weight during the same period (36 days). Halver et al (1957b) and Halver and Shanks (1960) reported similar observations with chinook salmon and sockeye salmon.

Shanks et al (1962) reported the development of scoliosis in 25% of rainbow trout fed on a tryptophan-deficient diet. However, this ill-effect was reversed by restoring tryptophan to the diet. Nose et al (1974) observed lordosis in carp receiving both dispensable and indispensable amino acid diets as well as in those on the control diet. They found that carp fed actively on the indispensable amino acid-deficient diet showed no signs of depression of appetite.

Mazid et al (1978) found with tilapia that the highest mortality occurred in fish fed on a leucine-deficient diet (25%). Lysine deficiency resulted in 25% mortality, followed by isoleucine (20%), arginine (10%) and histidine (5%). They also reported that 50% of the leucine-deficient population developed lordosis, which was also observed to some extent in the isoleucine-, arginine- and histidine-deficient fish. In this experiment, none of the groups of tilapia showed any loss in appetite during the entire feeding period. The authors concluded that the development of deficiency

symptoms are related to species and cannot always be attributed to dietary amino acid deficiency.

3. Utilisation of isomers and α-keto and α-hydroxy acids of amino acids

All amino acids isolated from the tissue proteins of animals were found to have the unchangeable L-configuration (Berg, 1959; D'Mello and Lewis, 1978). Whether the animals are capable of utilising the D-isomers, α-keto, and α-hydroxy analogues of amino acids for growth is important from the practical view of supplementation of commercial diets with D-forms of amino acids.

D'Mello and Lewis (1978) reviewed the evidence concerning the ability of different animal species to convert D-amino acids (dispensable or indispensable) to the L-form. This conversion involves the oxidative deamination of the D-isomer to the α-keto acid analogue, and, by a transamination reaction of the latter, the L-amino acid is produced. Both the rat and chick utilise the D-form of tryptophan, methionine, phenylalanine, leucine, valine and tyrosine, but not the D-form of lysine, threonine and isoleucine (D'Mello and Lewis, 1978). In the same review it was pointed out that chicks, in contrast to rats, were not able to utilise the D-forms of histidine and arginine.

The comparative aspects of α-keto and α-hydroxy acid utilisation by the growing rat have been discussed by D'Mello and Lewis (1978). The authors indicated that rats have the ability to

utilise both α-keto and α-hydroxy acids of histidine, isoleucine, leucine, methionine, phenylalanine and tryptophan, but have no ability to utilise those of lysine and threonine. The α-keto acid analogue of valine,in contrast to its α-hydroxy acid, was reported to be utilised by rats.

Utilisation of the various forms of methionine has been studied in channel catfish and in rainbow trout. Robinson et al (1978) demonstrated that both DL- and L-methionine are of equal efficiency in promoting optimum growth and optimum food utilisation in channel catfish, whereas methionine hydroxy analogue was only about 25% as effective as L-methionine in promoting growth. More recently, Kaushik and Luquet (1980) indicated that the L-form of methionine was more easily utilised by rainbow trout than the DL-form. Whether other species of fish are similar to rainbow trout and terrestrial vertebrates in their ability to utilise the L-isomer of methionine more effectively than the DL-form requires further investigation.

The toxic effects of D- and DL-amino acids have been indicated by Halver (1970). Little growth was obtained in salmonoids when three or more DL-forms of indispensable amino acids were incorporated in the diet. It may be concluded that salmonoids, as is the case with other animals, are unable to utilise excessive amounts of D-amino acids in the diet (Halver, 1976a,b).

E. Amino Acid Requirements: Quantitative Aspects

1. Methods of measuring amino acid requirements

a. Graded supplementation technique

This method is most frequently used in determining the indispensable amino acid requirements of growing animals (D'Mello, 1982). It involves the addition of graded supplements of the amino acid under study to a basal diet deficient in that amino acid. The crystalline form of the tested amino acid is usually employed.

When test diets are fed to the animal for a certain period of time (4-10 weeks), an asymptotic growth response curve should be obtained. The point at which no further increase in weight gain occurs (when the curve reaches its plateau) represents the requirement for that particular amino acid. In addition to growth, several other criteria, e.g. efficiency of food conversion, efficiency of protein deposition and plasma amino acids, may be measured to estimate the optimum requirement of the animals for the amino acid under test. According to the protein sources, two types of basal diets may be employed in this method:

(i) Semi-purified basal diets

This technique relies upon devising a basal diet which is low in the amino acid under test, but adequate in all other indispensable amino acids. In this technique, for instance, the dietary nitrogen required by the animal could be furnished by using

a combination of several natural protein sources.

Various test diets could be derived from this basal diet by incremental addition of the same amino acid under investigation, but in crystalline form.

Kaushik (1977 in Cowey, 1979) determined the arginine requirement of rainbow trout by feeding them diets containing different combinations of fish meal and zein. The results obtained with this technique were similar to those determined by measuring the tissue (blood and muscle) levels of free arginine in rainbow trout given different levels of dietary arginine. In the latter procedure, the requirement of rainbow trout was defined at the point when, due to further increase in arginine intake, an increase in the concentration of free arginine occurred.

More recently, Jackson and Capper (1982) estimated the arginine, lysine and methionine requirements of tilapia using test diets containing 400 g protein/kg, 50% of which was provided from a combination of groundnut, soya bean and fish meal, and the other 50% supplied from a mixture of synthetic amino acids. The basal diet was supplemented by the amino acid under study which was added at varying levels. The amino acid requirements were assessed from the growth of fish, efficiency of food conversion and net protein retention. It was indicated that this technique (using a combination of natural protein sources) might be more practical and useful in determining the amino acid requirements of fish that were unable to utilise the protein derived solely from synthetic amino

acids.

(ii) Purified basal diets

In this technique, the overall protein in the basal diets may be derived either from small amounts of purified protein such as casein or gelatin, and a larger amount of synthetic amino acids (DeLong et al, 1962; Klein and Halver, 1970; Wilson et al, 1978), or from a mixture of purified amino acids (Nose, 1978).

On the basis of the amino acid test diet designed by Halver (1957b), DeLong et al (1962) prepared a basal diet to determine the threonine requirements of chinook salmon. This diet consisted of casein, gelatin, an amino acid mixture, white dextrin, ∝-cellulose, corn oil, cod liver oil, carboxymethyl cellulose, minerals and vitamins. The levels of protein, casein and gelatin were adjusted to provide low levels of the amino acid under study. Higher levels of the amino acid under test were obtained by adding it, in crystalline form, to the basal diet at the expense of an equal amount of nitrogen included as L-alanine or L-proline. However, the dietary level at which maximum growth performance or efficiency of food conversion occurred was generally selected as the requirement. Amino acid requirements could also be estimated by plotting the weight gain of fish against the dietary concentrations of the amino acid under test. These procedures have been followed by several investigators in order to estimate the quantitative indispensable amino acid requirements of other cultured species of fish such as channel catfish (Wilson et al, 1977; Wilson et al,

1978; Wilson et al, 1980) and carp (Nose,1978).

Nose (1978) determined the requirements of carp for indispensable amino acids quantitatively by using an amino acid test diet. The amino acid under test was varied in the basal diet by replacing it with L-alanine on an iso-nitrogenous basis. These diets were fed to carp after being prepared as a paste and introduced into the experimental tank. The indispensable amino acid requirements of the carp were estimated by plotting the specific growth rate data obtained, using the equation of Brown (1957), against the concentrations of each individual amino acid examined. However, Nose (1978) assessed the requirements from these responses without any food intake measurements or statistical analysis.

b. Diet dilution technique

This method has been suggested by Fisher and Morris (1970) and involves the serial dilution of a high protein (summit) diet with an isoenergetic protein-free mixture (maize starch, ground oathulls, minerals and vitamins). The "summit" or undiluted ration supplies an excess level of all amino acids, e.g. 185% of assumed requirement (Fisher and Morris, 1970), except the amino acid under study, which is adjusted at a lower level of 145% of assumed requirement (Fisher and Morris, 1970). When the summit diet is diluted with the protein-free mixture, dietary amino acid balance remains constant, and the amino acid under test is first-limiting at all levels of dilution. This method relies on interpreting

responses to different levels of dilution as responses to the first-limiting amino acid and not to changing protein levels in the diets. Fisher and Morris (1970) concluded that the dilution technique is superior to the graded supplementation method.

c. Carcass composition

This method has been employed by Williams et al (1954) in studies with rats, chicks and pigs. More recently, Ogino (1980) followed the same procedure to determine the amino acid requirements of rainbow trout and carp.

This technique relies on an initial assessment of the requirement, as grams per day, for a single amino acid, e.g. lysine. The requirements of other amino acids could be estimated from the proportion (ratios) existing between the indispensable amino acids and lysine in the carcass of the animal. Two reasons (Williams et al, 1954) for selecting lysine as a standard for calculating the requirements are : (i) its recognised importance as an essential dietary nutrient for animal growth; (ii) its major role as an essential component of protein synthesis.

d. Limitation of the methods

D'Mello (1982) compared two experimental methods, the graded supplementation and the dilution techniques of determining amino acid requirements, to examine recent criticisms (Fisher and Morris, 1970; Gous, 1980 in D'Mello, 1982) levelled at the supplementation

technique and he concluded that both methods were equally acceptable, but that the graded supplementation method may offer advantages in the case of testing the interaction or pairs or groups of amino acids, e.g. lysine and arginine or leucine, isoleucine and valine.

The determined requirements for indispensable amino acids by carcass composition is entirely dependent on the accuracy of the amino acid analysis. As indicated earlier, certain amino acids, e.g. methionine and cystine, cannot be measured accurately (Cowey and Sargent, 1972), and consequently the requirements of the animal for such amino acids estimated by this method, could not be justified. Furthermore, this method seems to ignore amino acid requirements for other purposes, e.g. maintenance (Walton et al, 1982).

As is clear from the foregoing discussion, the estimation of amino acid requirements by nutritional experiment is of more practical value than assessment by carcass analysis. However, since certain fish species are unable to utilise high proportions of free amino acids in their diet, it may be concluded that the graded supplementation technique employing semi-purified diets offers the most suitable system of determining the indispensable amino acid requirements of fish.

e. Methods of expressing amino acid requirements

Amino acid requirements are conventionally expressed as percentages or as grams per kilogram of diet. These expressions are convenient for dietary formulation, but may present a false picture when attempts are made to resolve those factors which genuinely influence amino acid requirements and those which simply alter voluntary food intake (D'Mello, 1978).

Growth increments resulting from a limiting intake of an amino acid will depend upon its level in the ration and on the food intake of the animal, since food intake would be expected to fluctuate. D'Mello (1976) used such an approach in a comparative study of sulphur amino acid requirements of growing chicks (D'Mello, 1973) and young turkeys (D'Mello, 1976). He demonstrated that the requirements of chicks and turkeys for methionine and cystine for a given rate of growth were similar when viewed in terms of daily intake (Fig. 4). This also indicated the similarities in utilisation of the sulphur amino acids by these animals.

The above findings emphasise the need for confirmatory feeding trials in fish with appropriate test diets, careful amino acid analysis and measurement of food intake. From such investigations, it may be possible to define the similarities in the daily intake of methionine and cystine required to promote a given growth rate in various species of fish.

2. Requirements for dietary indispensable amino acids

Quantitative data on indispensable amino acid requirements has

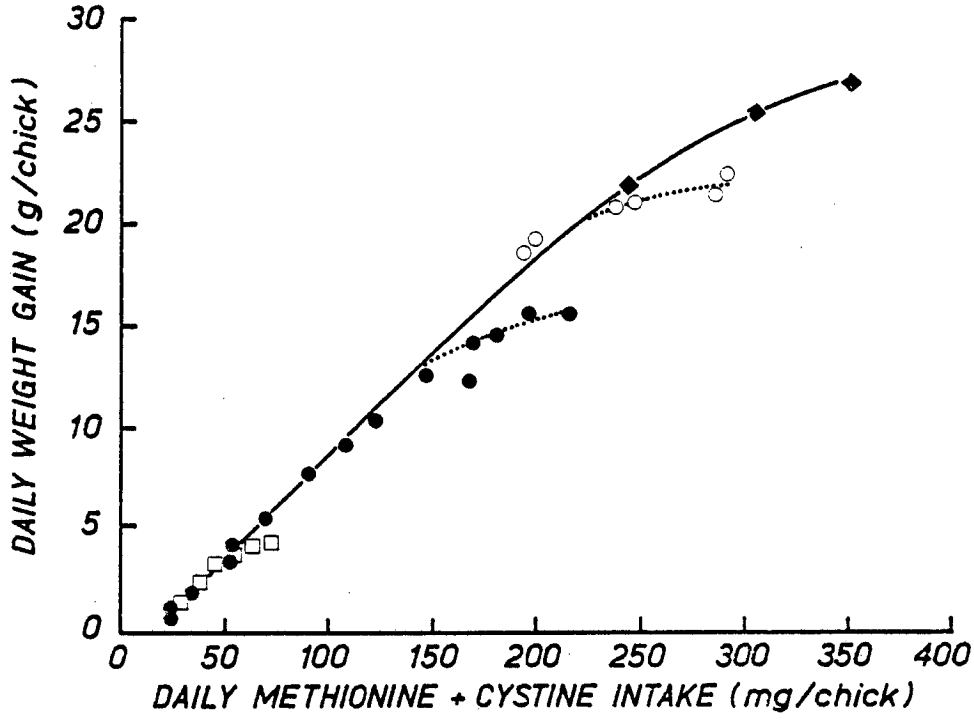


Fig. 4 Daily weight gain (g/animal) of growing rats (□), slow-growing chicks (●), fast-growing chicks (○) and young turkeys (■) in relation to daily methionine + cystine intake (mg/animal). (Adopted from D'Mello, 1976)

been reported for several species of fish, e.g. chinook salmon, (Halver et al, 1958; Halver et al, 1959; Chance et al, 1964; Halver 1965; Klein and Halver, 1970), channel catfish (Wilson et al, 1977; Harding et al, 1977; Wilson et al, 1978), carp (Nose, 1978; Ogino, 1980) eel (Arai and Nose, unpublished data cited in NAS/NRC, 1977a) and rainbow trout (Ogino, 1980). The data shown in the following tables (4-13) suggests that requirements, as g/kg diet, for certain indispensable amino acids, differ between fish species. Amino acid requirements of carp and rainbow trout, as reported by Ogino (1980), were found to be similar for both species. Due to the fact that little is known about the amino acid requirements of fish in terms of intake, similarities in the amino acid requirements of various species of fish cannot be demonstrated as terrestrial animals (D'Mello, 1975; D'Mello and Emmans, 1975; D'Mello, 1976; 1978).

a. Arginine

The arginine requirements of different fish species are shown in Table 4. In terms of dietary concentrations, the requirements of both carp and eel are similar (17.0 g/kg). This value is noticeably higher than that of rainbow trout (14.0 g/kg) and carp (15.2 g/kg) as reported by Ogino (1980). The requirement of channel catfish (10 g/kg) seems to be the lowest from among the various species of fish. Recently, Jackson and Capper (1982) have shown that the requirement of tilapia for arginine was less than 15.9 g/kg. However, when expressed as g/kg dietary protein, the arginine requirement of carp (34.0 g/kg, Nose, 1978), is marginally

TABLE - 4

Arginine requirements of carp, chinook salmon, rainbow trout, channel catfish, eel and tilapia.

Fish species	Arginine requirement		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	17.0	34.0	Graded supplementation (purified diet)	Nose (1978)
Carp	15.2	38.0	Carcass composition	Ogino (1980)
Chinook salmon	24.0	60.0	Graded supplementation (purified diet)	Klein and Halver (1970)
Rainbow trout	14.0	35.0	Carcass composition	Ogino (1980)
Channel catfish	10.3	42.9	Graded supplementation (purified diet)	Robinson et al (1981)
Eel	17.0	39.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)
Tilapia	<15.9	<39.7	Graded supplementation (semi-purified diet)	Jackson and Capper (1982)

lower than that found for rainbow trout. This estimate, however, is considerably lower than the value (38.0 g/kg) reported for carp by Ogino (1980), eel (39.0 g/kg), tilapia (> 39.7 g/kg), channel catfish (42.9 g/kg) and chinook salmon (60.0 g/kg).

b. Histidine

When expressed in terms of dietary concentrations, the histidine requirements of carp (Nose, 1978) and eel were found to be the same (8 g/kg). This estimate is higher than that reported for carp (5.6 g/kg, Ogino, 1980), rainbow trout (6.4 g/kg) and chinook salmon (7 g/kg), but about twice that amount reported for channel catfish (3.7 g/kg) as shown in Table 5. When considered as a proportion of dietary protein, the arginine requirement of carp (Nose, 1978) is (21.0 g/kg) considerably higher than that of channel catfish (15.4 g/kg), rainbow trout (16.0 g/kg), chinook salmon (18.0 g/kg) and eel (19.0 g/kg). Again, the histidine requirement of carp determined by Nose (1978) is much higher than the value (14.0 g/kg) reported by Ogino (1980) for the same species.

c. Isoleucine

As isoleucine, leucine and valine are structurally similar, and because there is a specific interaction between these amino acids, the dietary isoleucine requirements of animals are affected by the levels of dietary leucine and valine (see Section I-E-3-d).

TABLE - 5

Histidine requirements of carp, chinook salmon, channel catfish and eel.

Fish species	Histidine requirement		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	8.0	21.0	Graded supplementation (purified diet)	Nose (1978)
Carp	5.6	14.0	Carcass composition	Ogino (1980)
Chinook salmon	7.0	18.0	Graded supplementation (purified diet)	Klein and Halver (1970)
Rainbow trout	6.4	16.0	Carcass composition	Ogino (1980)
Channel catfish	3.7	15.4	Graded supplementation (purified diet)	Wilson et al (1980)
Eel	8.0	19.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)

The dietary isoleucine requirement of eel (15.0 g/kg) was found to be higher than that of carp (9.2-10.0 g/kg), chinook salmon (9.0 g/kg) and rainbow trout (9.6 g/kg), as is indicated in Table 6. Channel catfish were shown to require (6.2 g/kg) less than 50% of that reported for eels. When these requirements are expressed as a proportion of dietary protein, carp (Nose, 1978) need a marginaly higher amount (25.8 g/kg) than that (25.0 g/kg) required by channel catfish. These values, however, are higher than those reported for chinook salmon (22.0 g/kg) carp (23.0 g/kg, Ogino, 1980) and rainbow trout (24.0 g/kg), and much lower than those (36.0 g/kg) reported for eel.

d. Leucine

The data presented in Table 7 illustrate the leucine requirements of several species of fish. As a function of dietary concentrations, the leucine requirements of rainbow trout (17.6 g/kg), eel (17.0 g/kg) and chinook salmon (16.0 g/kg) are considerably higher than those of channel catfish (8.4 g/kg). As can be seen from Table 7, the latter value is also much lower than those (13.0 and 16.4 g/kg) estimates reported for carp by separate authors, Nose (1978) and Ogino (1980) respectively. When expressed as a proportion of dietary protein, the leucine requirement of rainbow trout (44.0 g/kg) is considerably higher than that of carp (33.0 g/kg), channel catfish (35.0 g/kg) and chinook salmon (39.0 g/kg) and eel (41.0 g/kg).

e. Lysine

TABLE - 6

Isoleucine requirements of carp, chinook salmon, rainbow trout, channel catfish and eel.

Fish species	Isoleucine requirement		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	10.0	25.0	Graded supplementation (purified diet)	Nose (1978)
Carp	9.2	23.0	Carcass composition	Ogino (1980)
Chinook salmon	9.0	22.0	Graded supplementation (purified diet)	Chance et al (1964)
Rainbow trout	9.6	24.0	Carcass composition	Ogino (1980)
Channel catfish	6.2	25.8	Graded supplementation (purified diet)	Wilson et al (1980)
Eel	15.0	36.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)

TABLE - 7

Leucine requirements of carp, chinook salmon, rainbow trout, channel catfish and eel.

Fish species	Leucine requirement		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	13.0	33.0	Graded supplementation (purified diet)	Nose (1978)
Carp	16.4	41.0	Carcass composition	Ogino (1980)
Chinook salmon	16.0	39.0	Graded supplementation (purified diet)	Chance et al (1964)
Rainbow trout	17.6	44.0	Carcass composition	Ogino (1980)
Channel catfish	8.4	35.0	Graded supplementation (purified diet)	Wilson et al (1980)
Eel	17.0	41.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NCR (1981)

As shown in Table 8, the dietary lysine requirements of carp (21.2 g/kg) and rainbow trout (21.2 g/kg) reported by Ogino (1980) are quite similar, but these estimates are slightly lower than the value (22.0 g/kg) published by Nose (1978) for carp. However, the latter estimate is higher than that of tilapia (16.2 g/kg) and about twice that reported (12.3 g/kg) for catfish. The eel was shown to require 20 g/kg of dietary lysine, which is marginally lower than that of the rainbow trout and carp (Ogino, 1980). The requirement of channel catfish for dietary lysine (12.3 g/kg) tends to be lower than that of other species of fish. When these requirements are considered in terms of g/kg of dietary protein, tilapia appear to have the lowest requirement for lysine (40.5 g/kg), and carp (Nose, 1978), the highest (57.0 g/kg). However, the requirement of carp for lysine is considerably higher than that of eel (48.0 g/kg), chinook salmon (50.0 g/kg) and rainbow trout (53.0 g/kg). Recently Robinson et al (1980b) estimated the lysine requirement of channel catfish fingerling to be about 51 g/kg dietary protein. This estimate confirms the value of 51 g/kg dietary protein previously reported by Wilson et al (1977).

f. Sulphur amino acids (methionine and cystine)

The synthesis of cystine from methionine is believed to occur in fish (Mertz, 1969) as in other animals, e.g. rats (Sowers et al, 1972; Stockland et al, 1973), chicks (Graber et al, 1971a,b,c; Graber and Baker, 1971; Sasse and Baker, 1974; D'Mello, 1978; Featherston and Rogler, 1978; Wheeler and Latshaw, 1981) and turkeys (D'Mello, 1976). Thus, only methionine is identified as an

TABLE - 8

Lysine requirements of carp, chinook salmon, rainbow trout, channel catfish, eel and tilapia.

Fish species	Lysine requirement		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	22.0	57.0	Graded supplementation (purified diet)	Nose (1978)
Carp	21.2	53.0	Carcass composition	Ogino (1980)
Chinook salmon	20.0	50.0	Graded supplementation (purified diet)	Halver et al (1958)
Rainbow trout	21.2	53.0	Carcass composition	Ogino (1980)
Channel catfish	12.3	51.3	Graded supplementation (purified diet)	Wilson et al (1977)
Channel catfish	15.0	51.0	Graded supplementation (purified diet)	Robinson et al (1980b)
Eel	20.0	48.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)
Tilapia	16.2	40.5	Graded supplementation (semi-purified diet)	Jackson and Capper (1982)

indispensable amino acid.

Early reports (Womack and Rose, 1941) on the replacement value of cystine for methionine have shown that cystine could supply 16.7% of methionine in rats. More recently, Sowers et al (1972) have found that cystine can contribute up to 64% of the total sulphur amino acid requirement of rats.

Several different sets of results are available on the replacement value of cystine for methionine in chicks. These estimates range between 52% and 56% (Sasse and Baker, 1974; Graber and Latshaw, 1981; Baker et al, 1969).

In comparison with endothermic animals, little work has been carried out with fish to determine the replacement value of cystine for methionine. It is believed to follow a similar pattern to that of terrestrial vertebrates. Harding et al (1977) have shown that cystine could provide 60% of the methionine requirement of channel catfish. More recently, Jackson and Capper (1982) published an estimate for tilapia similar to that suggested in the case of channel catfish. However, both studies, Harding et al (1977) and Jackson and Capper (1982), were not designed to investigate the replacement value of cystine for methionine on the basis of factorial arrangements between both sulphur-containing amino acids. More research needs to be conducted to establish the maximum amount of the total sulphur amino acid needs of fish that can be provided by cystine.

The requirement of chinook salmon for methionine was found to be about 6 g/kg diet (or 40 g/kg dietary protein) in the presence of 10 g cystine/kg diet (Halver et al, 1959). At a low cystine level (0.5 g/kg diet), chinook salmon failed to achieve maximal growth with a methionine level of 16 g/kg diet.

In general, salmonoids appear to require about 15 g/kg diet of the total sulphur amino acids, with best growth observed when 10 g/kg diet of cystine and 5 g/kg diet of methionine were fed (Halver et al, 1959). A recent report (Ogino, 1980) indicates that rainbow trout require about 6.3 g methionine/kg diet in the presence of 3.2 g cystine/kg diet. These values, however, are much lower than those reported for chinook salmon (Halver et al, 1959). More recently, Walton et al (1982) demonstrated the methionine requirement of rainbow trout to be 5-10 g/kg diet in the absence of cystine, but 5 g/kg diet in the presence of 20 g cystine/kg diet.

Harding et al (1977) demonstrated with channel catfish that the methionine requirement in the absence of cystine is about 5.6 g/kg diet (23.4 g/kg dietary protein). They also indicated that the replacement value of cystine for methionine on an equimolar sulphur basis was 60%.

Nose (1978) determined the methionine requirement of carp to be 12 g/kg diet (31 g/kg dietary protein) in the absence of cystine. In the presence of 20 g cystine/kg diet, the methionine requirement was found to be 8.1 g/kg diet or 21 g/kg dietary protein. A recent estimate (Ogino, 1980) for the methionine requirement of carp, in

the presence of 3.2 g cystine/kg diet, is 6.3 g/kg diet or 18 g/kg dietary protein. This estimate is much lower than that reported for carp by Nose (1978) as shown in Table 9.

Cowey (1979) reviewed evidence that the relative amounts of cystine and methionine in the eel diet were 9 and 10 g/kg respectively. More recently, tilapia (Jackson and Capper, 1982) were found to have the lowest methionine requirement (<5.3 g/kg diet) in the presence of 7.4 g cystine/kg diet. This result indicates that the dietary methionine was not sub-optimal even at a level of 5.3 g/kg, and therefore the requirement could not be estimated exactly. However, even this value (5.3 g/kg diet) is much lower than that reported for carp, (Nose, 1978).

It is clear from the published data concerning the requirements of fish for sulphur amino acids,that as with endothermic animals, they are able to convert methionine to cystine. However, most of the data available in this area indicates that no in-depth studies have been conducted on the nutritional significance of methionine to cystine conversion.

A survey of the relevant literature shows that no clear relationship could be established between dietary cystine levels and methionine requirements of different species of fish (Fig. 5). This is attributed to lack of methionine requirement data at different levels of dietary cystine. However, the studies of Harding et al (1977) indicate a linear decrease in methionine requirements for channel catfish. At first glance it would appear

TABLE - 9

Methionine requirements in relation to cystine level of carp, chinook salmon, rainbow trout, channel catfish, eel and tilapia.

Fish species	Methionine requirement		Cystine content	Technique used	Reference
	g/kg diet	g/kg dietary protein	g/kg diet		
Carp	12.0	31.2	0.0	Graded supplementation (purified diet)	Nose (1978)
	8.1	21.0	20.0		
Carp	6.3	18.0	3.2	Carcass composition	Ogino (1980)
Chinook salmon	6.0	15.0	10.0	Graded supplementation (purified diet)	Halver et al (1959)
Rainbow trout	6.3	18.0	3.2	Carcass composition	Ogino (1980)
Rainbow trout	5-10	10-20	0.0	Graded supplementation (purified diet)	Walton et al (1982)
	10.0	20.0	20.0		
Channel catfish	5.6	23.4	0.0	Graded supplementation (purified diet)	Harding et al (1977)
Eel	9.0	21.4[a]	10.0	Graded supplementation (purified diet)	Cowey and Sargent (1979)
Tilapia	<5.3	<13.3	7.4	Graded supplementation (semi-purified diet)	Jackson and Capper (1982)

[a] calculated as 420g dietary protein/kg (NAS/NRC, 1981)

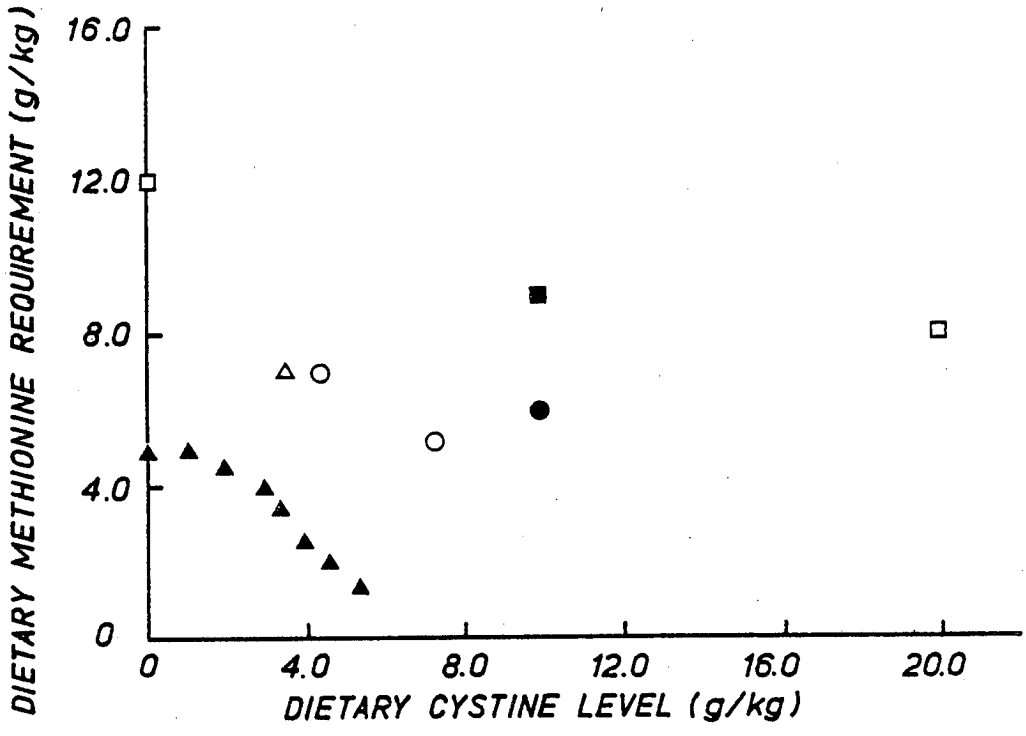


Fig. 5 The relationship between dietary methionine requirement (g/kg) and dietary cystine level (g/kg) in rainbow trout (△), tilapia (○), carp (□), chinook salmon (●), channel catfish (▲) and eel (■). Data for rainbow trout from Ogino (1980), for tilapia from Jackson and Capper (1982), for carp from Nose (1978), for chinook salmon from Halver et al (1959), for channel catfish from Harding et al (1977) and for eel from Cowey and Sargent (1979).

that this pattern is different from that recorded for terrestrial vertebrates. In the latter group of animals, exemplified by chicks and rats (Fig. 6), methionine requirements fall linearly with increasing cystine concentrations in the diet up to a point represented by about 30 g cystine/kg dietary protein. Beyond this point, methionine requirements are independent of cystine concentrations in the diet. These patterns are consistent with the biochemical conversion of methionine to cystine and with the nutritional indispensability of methionine.

It is clear that much work needs to be conducted on methionine to cystine conversion in fish and particularly in carp. The substantial differences in methionine requirements of various species of fish (Table 9) may be attributed to the varying proportions of cystine in the basal diets used.

g. Phenylalanine and Tyrosine

Phenylalanine and tyrosine both contain an aromatic ring which cannot be manufactured by animals. Only phenylalanine is classified as an indispensable amino acid since most animals are able to convert phenylalanine to tyrosine (Mertz et al, 1954; Armstrong, 1955; Almquist, 1959; Stockland et al, 1971; Sasse and Baker, 1972). For example, young albino rats were found to require about 12 g phenylalanine/kg diet in the absence of tyrosine, but in the presence of excess tyrosine, this requirement was reduced to 6 g/kg diet (Armstrong, 1955). Such a reduction in phenylalanine requirement demonstrates the ability of animals to synthesise

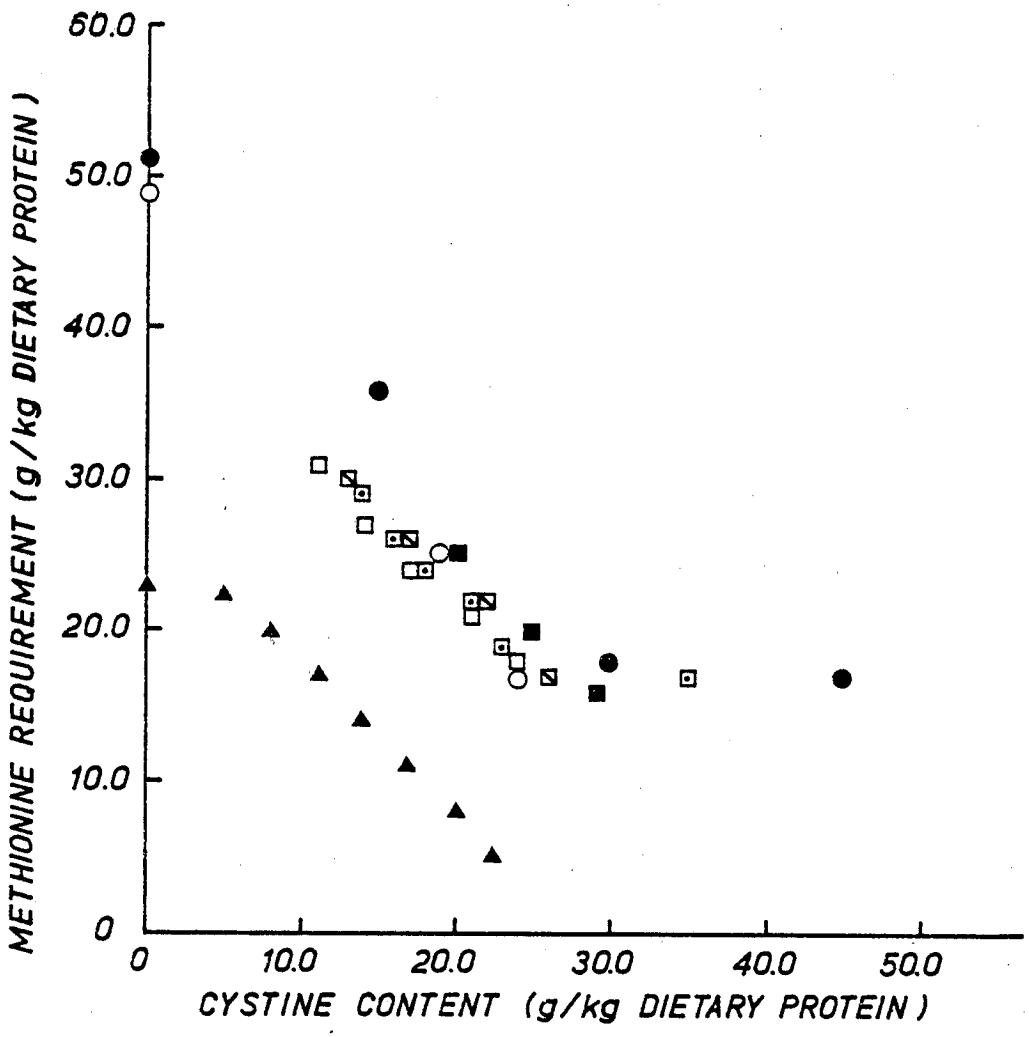


Fig. 6 The relationship between dietary methionine requirement (g/kg dietary protein) and dietary cystine level (g/kg dietary protein) in rats [(●), Sowers et al, 1972; (O), Stockland et al, 1973], chicks [(⊡), Wheeler and Latshaw, 1981; (□,▨,■), Graber et al, 1971b] and channel catfish [(△), Harding et al, 1977].

tyrosine from phenylalanine. Recently, it has been indicated that for the chick, at least 58% of the combined aromatic amino acid requirement must be supplied as phenylalanine (D'Mello, 1978).

The quantitative phenylalanine requirement of chinook salmon was estimated to be approximately 17 g/kg dry diet (41.5 g/kg dietary protein) in the presence of 4 g tyrosine/ kg diet (Chance et al, 1964). Halver (1976a) indicated that about one third of the phenylalanine requirement for growth could be provided by tyrosine. However, Ogino (1980) found the dietary phenylalanine requirement of rainbow trout to be 12.4 g/kg in the presence of 8.4 g dietary tyrosine/kg. On the basis of total aromatic amino acid requirements, values obtained by Ogino (1980) are in close agreement with those reported for chinook salmon by Chance et al (1964).

Nose (1978) demonstrated that the phenylalanine requirement of carp depended upon the level of dietary tyrosine. It was found that dietary phenylalanine of up to 15 g/kg in the absence of tyrosine could not sustain the growth of carp, but that a rapid growth response was observed with phenylalanine additions of up to 25 g/kg diet. Further additions of phenylalanine did not stimulate the growth of carp. Nose, however, (1978) assumed the requirement of carp for phenylalanine to be 25 g/kg diet (65 g/kg dietary protein) in the presence of tyrosine (28.6 g/kg dietary protein or 10 g/kg diet), which decreased to 13.1 g/kg diet when 10 g dietary tryosine was included. In the same study, the conversion rate of phenylalanine to tyrosine was suggested to be about 60%. The

phenylalanine requirement of carp was determined by Ogino (1980) to be 11.6 g/kg diet in the presence of 8 g tyrosine/kg diet, which is lower than that reported by Nose (1978) as shown in Table 10.

Robinson et al (1980a) conducted a series of experiments in order to determine the aromatic amino acid requirement, the replacement value for phenylalanine and the phenylalanine requirement for fingerling channel catfish under conditions of adequate tyrosine level. They found that the total dietary aromatic amino acid requirement was approximately 12 g/kg dry diet (50 g/kg dietary protein), of which about 50% could be supplied by tyrosine. The quantitative phenylalanine requirement was found to be about 4.9 g/kg diet (20.4 g/kg dietary protein) in the presence of adequate level of tyrosine (6.0 g/kg diet). However, the requirements of channel catfish for phenylalanine and tyrosine were found to be slightly lower (Table 10) to that of chinook salmon, but higher than that of eels and lower than that reported for carp.

h. Threonine

The threonine requirements of carp (Nose, 1978) and eel are similar (15.0 g/kg diet). As shown in Table 11, this estimate is slightly higher than that reported for carp (13.2 g/kg) and rainbow trout (13.6 g/kg) by Ogino (1980). The latter values are considerably higher than those found for channel catfish (5.3 g/kg) and chinook salmon (9.0 g/kg diet).

When this requirement is expressed as g/kg dietary protein,

TABLE - 10

Phenylalanine requirements in relation to tyrosine level of carp, chinook salmon, rainbow trout, channel catfish and eel.

Fish species	Phenylalanine requirement		Tyrosine content	Technique used	Reference
	g/kg diet	g/kg dietary protein	g/kg diet		
Carp	25.0	65.0	0.0	Graded supplementation (purified diet)	Nose (1978)
	13.1	34.0	10.0		
Carp	11.6	29.0	8.0	Carcass composition	Ogino (1980)
Chinook salmon	17.0	41.5	4.0	Graded supplemention (purified diet)	Chance et al (1964)
Rainbow trout	12.4	31.0	8.4	Carcass composition	Ogino (1980)
Channel catfish	4.9	20.0	6.0	Graded supplementation	Robinson et al (1980a)
Eel	22.0	45.0	0.0	Graded supplementation	Arai and Nose cited in Cowey and Sargent (1979)

TABLE - 11

The requirements of carp, chinook salmon, rainbow trout, channel catfish and eel for threonine.

Fish species	Threonine requirement		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	15.0	39.0	Graded supplementation (purified diet)	Nose (1978)
Carp	13.2	33.0	Carcass composition	Ogino (1980)
Chinook salmon	9.0	22.0	Graded supplementation (purified diet)	Halver et al (1958)
Rainbow trout	13.6	34.0	Carcass composition	Ogino (1980)
Channel catfish	5.3	22.1	Graded supplementation (purified diet)	Wilson et al (1978)
Eel	15.0	36.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)

chinook salmon and channel catfish were found to have similar needs. However, the threonine requirement (39.0 g/kg) of carp (Nose, 1978) is highest from among those species examined.

i. Tryptophan

In many species of animals (Krehl et al, 1945; Almquist, 1959; Harper, 1964), the tryptophan requirement is dependent upon the dietary concentration of niacin since, these animals have the ability to convert tryptophan to niacin. Studies with fish have so far shown that brook trout (Salvelinus fontinalis), (Poston and DiLorenzo, 1973) or salmonoids in general (Poston and Combs, 1980) and channel catfish (Wilson et al, 1978) are unable to convert tryptophan to niacin.

Of the indispensable amino acids, tryptophan was only required in the smallest quantities to induce maximum growth of fish (Table 12). The tryptophan requirement of eel (4.0 g/kg diet or 10.0 g/kg dietary protein) was found to be about twice that required for maximum growth of chinook salmon and rainbow trout (2.0 g/kg diet or 5.0 g/kg dietary protein). Channel catfish, chinook salmon and rainbow trout appeared to require the same amount of tryptophan when the requirement was expressed as g/kg dietary protein. The tryptophan requirement, as g/kg diet or g/kg dietary protein, of carp reported by Nose (1978) is less than that of eel and higher than that of chinook salmon, rainbow trout and channel catfish.

The lower dietary tryptophan requirements of certain fish

TABLE - 12

The dietary tryptophan requirements of carp, chinook salmon, rainbow trout, channel catfish and eel.

Fish species	Tryptophan requirement		Dietary niacin	Technique used	Reference
	g/kg diet	g/kg dietary protein	g/kg diet		
Carp	3.0	8.0	0.80	Graded supplementation (purified diet)	Nose (1978)
Carp	2.4	6.0		Carcass composition	Ogino (1980)
Chinook salmon	2.0	5.0	0.75	Graded supplementation (purified diet)	Halver (1965)
Rainbow trout	2.0	5.0		Carcass composition	Ogino (1980)
Channel catfish	1.2	5.0	0.13	Graded supplementation (purified diet)	Wilson et al (1978)
Eel	4.0	10.0		Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)

species, as compared with other terrestrial vertebrates (see I-E-2-i), may indicate the inability of these fish to convert tryptophan to niacin (Wilson et al, 1978). Whether or not tryptophan is an adequate precursor of dietary niacin for other fish species, as in other animals, needs to be investigated.

j. Valine

As already indicated, valine, isoleucine and leucine are structurally similar, and therefore the valine requirement of animals is affected by the dietary concentrations of isoleucine and leucine (see Section I-E-3-d).

The valine requirement of eel (15.0 g/kg diet) is marginally higher than that found for carp (14.0 g/kg), but considerably higher than that reported for channel catfish (7.1), rainbow trout (12.4) and chinook salmon (13.0 g/kg diet) as shown in Table 13.

When these estimates are considered as a proportion of dietary protein, the requirements of carp and eel (36.0 g/kg) are similar. However, this value is much higher than that suggested for channel catfish (29.6), rainbow trout (31.0) and chinook salmon (32.0 g/kg).

As shown in Tables 4-13, fish species differ widely in their requirements, as g/kg diet or g/kg dietary protein, for certain amino acids. These dissimilarities could be attributed to the different techniques employed by different investigators, and to

TABLE - 13

The requirements of carp, chinook salmon, rainbow trout, channel catfish and eel for valine.

Fish species	Valine requirements		Technique used	Reference
	g/kg diet	g/kg dietary protein		
Carp	14.0	36.0	Graded supplementation (purified diet)	Nose (1978)
Carp	11.6	29.0	Carcass composition	Ogino (1980)
Chinook salmon	13.0	32.0	Graded supplementation (purified diet)	Chance et al (1964)
Rainbow trout	12.4	31.0	Carcass composition	Ogino (1980)
Channel catfish	7.1	29.6	Graded supplementation (purified diet)	Wilson et al (1980)
Eel	15.0	36.0	Graded supplementation (purified diet)	Arai and Nose cited by NAS/NRC (1981)

several other factors that influence amino acid requirements. These discrepancies suggest the need for confirmatory feeding experiments with appropriate test diets, careful amino acid analysis and measurement of intake. Furthermore, measurement of amino acid intake needs particular attention as the requirements of various animal species for several amino acids have been shown to be similar, even under different experimental conditions, when the intake of amino acid was measured (D'Mello, 1975; D'Mello and Emmans, 1975; D'Mello, 1976; 1978).

3. Factors affecting amino acid requirements

Various factors influence the amino acid requirements of domesticated animals. Among these are genetic factors, environmental temperature, and several nutritional factors such as dietary levels of protein and energy and amino acid imbalance. Few studies have so far been made to estimate the impact of these factors on the amino acid requirements of fish.

a. Genetic factors

Mertz (1969) compared the amino acid requirements of chinook salmon with those of the chick, pig and rat, and showed that these animals differed in their needs for certain amino acids. Recent published data concerning the amino acid requirements of fish is compared with that of other animals in Table 14.

TABLE - 14

Amino acid requirements (g/kg diet or g/kg dietary protein) of different animals.

Amino acid	Carp		Chinook salmon		Channel catfish		Eel		Tilapia		Rat		Pig		Chick		Turkey	
	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein	g/kg diet	g/kg dietary protein
Arginine	17	44.1	24	60.0[1]	10.3	42.9[6]	17	40.5	<15.9	<39.7	2	10.5	2	15.4	11	61.1	17.5	61.7[13]
Histidine	8	20.8	7	17.5[1]	3.7	15.4[7]	8	19.0			4	21.0	2	15.4	3	16.7	5.0	17.9[15]
Isoleucine	10	26.0	9	22.0[2]	6.2	25.8[7]	15	35.7			5	38.5	6	46.1	8	44.4	8.4	29.9[12]
Leucine	13	33.8	16	39.0[2]	8.4	35.0[7]	17	40.5			9	45.0	6	46.1	12	66.7	15.5	54.8[12]
Lysine	22	57.1	20	50.0[3]	12.3	51.3[8]	20	47.6	16.2	40.5	10	52.6	6.5	50.0	11	61.1	14.2	50.5[13]
Methionine	12	31.2	16[a]	40.0[4]	5.6	23.3[9]	21[a]	50.0	<5.3	<13.3	6	30.0	6	30.0	8	44.4	8.3	30.6[14]
Phenylalanine	25	64.9	21[b]	51.2[2]	12.0[b]	50.0[10]	22	52.4			9	52.9	4.6	35.4	13	72.2	8.0	28.6[15]
Threonine	15	39.0	9	22.5[3]	5.3	22.1[11]	15	35.7			5	31.2	4	30.8	6	33.3	9.0	32.1[15]
Tryptophan	3	7.8	2	5.0[5]	1.2	5.0[11]	4	9.5			2	10.5	2	8.0	2	11.1	2.2	7.9[15]
Valine	14	36.4	13	32.5[2]	7.1	29.6[7]	15	35.7			4	30.8	4	30.8	8	44.4	12.1	44.1[12]
References:	Nose (1978)		[1]Klein and Halver (1970) [2]Chance et al (1964) [3]Halver et al (1958) [4]Halver et al (1959) [5]Halver (1965)		[6]Robinson et al (1981) [7]Wilson et al (1980) [8]Wilson et al (1977) [9]Harding et al (1977) [10]Robinson et al (1980) [11]Wilson et al (1978)		Arai and Nose (unpublished data, cited by NAS/NRC, 1981; Cowey, 1979)		Jackson and Capper (1982)		Mertz (1969)		Mertz (1969)		Mertz (1969)		[12]D'Mello (1975) [13]D'Mello and Emmans (1975) [14]D'Mello (1976) [15]ARC (1975)	

[a] plus cystine
[b] plus tyrosine

NAS/NRC (1981) suggested that expressing the amino acid requirements as a proportion of dietary protein would be a useful technique in comparative studies. Accordingly, in this section the amino acid requirements of various animals have been compared.

As g/kg dietary protein, the arginine requirements of fish and birds (40-62 g/kg) are noticeably higher than those of mammals (11-15 g/kg). This could be due to the lack of a urea cycle in fish and birds, which in growing mammals provides about 75% of the arginine required for growth (Mertz, 1969).

The histidine requirements of carp (20.8 g/kg) and and rats (21 g/kg) are similar, but higher than those reported for other species of animals, e.g. chinook salmon (17.5 g/kg), turkeys (18.0 g/kg) and eels (19.0 g/kg).

The isoleucine requirements of the chick (44 g/kg) and young pig (46 g/kg) are considerably higher than those of fish (22-36 g/kg). Isoleucine and leucine interaction and the different ratios of these amino acids used by the investigators may contribute to the wide variation in requirements. The reported data concerning the requirements for leucine in the chick (67 g/kg) and turkey (54.8 g/kg) are usually higher than other species, possibly due to formation of feathers (Metz, 1969).

The carp is the only fish that requires relatively high (57 g/kg) amounts of lysine. However, this estimate is lower than that found for the chick (61 g/kg).

Among those species tested, channel catfish have the lowest requirement for methionine (23.4 g/kg). The requirements of the rat (30 g/kg), young pig (30 g/kg) and carp (31 g/kg) for methionine, in the absence of cystine, are relatively similar and these estimates are lower than those for the chick (44 g/kg).

The requirement of carp (Nose, 1978) for methionine and cystine was therefore found to be much higher (73.6 g/kg) than that reported for chinook salmon and rainbow trout (40 g/kg) Again, the value reported for carp is more than three times that (>20.6 g/kg) reported for tilapia, and even higher than that reported for turkeys (30.6 g/kg).

The phenylalanine requirement (72 g/kg) of the chick, in the absence of tyrosine, was found to be higher than that reported for other animals. In the presence of tyrosine, chinook salmon, channel catfish and rats required approximately the same amount (51, 50 and 53 g/kg respectively).

The threonine requirement (39 g/kg) of carp was reported to be higher than that of other species, but this estimate was lower than that for the eel (36 g/kg).

The eel, rat and chick have approximately similar requirements (9.5, 10 and 11 g/kg) for tryptophan which are considerably higher than those of chinook salmon and channel catfish (5 g/kg). However, the carp and pig have similar requirements for tryptophan (8 g/kg), but this value is slightly lower than those reported for

the eel, rat and chick.

The valine requirements of carp and eel are similar (36 g/kg), but this estimate is noticeably lower than those for the chick (44 g/kg) and turkey (44.1 g/kg), and slightly higher than those recommended for chinook salmon (32 g/kg), channel catfish (29.6 g/kg), the rat (31 g/kg) and the pig (31 g/kg).

From the data presented in this section, it should be emphasised that the requirement for certain indispensable amino acids differs greatly from one species to another when expressed in terms of dietary protein concentrations. Expressing these requirements in this manner, however, could be mainly responsible for the reported differences.

b. Environmental temperature

The only available data on the amino acid requirements of fish in relation to water temperature are those of DeLong et al, (1962). These researchers demonstrated that the two temperatures tested (8^{o} and 15^{o}C) did not affect the threonine requirement, as g/kg diet, of chinook salmon when the fish were fed a constant dietary protein concentration (500 g/kg). They suggested, however, that the threonine requirement could shift upwards in warmer waters if the fish were fed on a higher protein diet to satisfy their increased needs.

Experience with other animals has shown that environmental

temperature has an important effect in influencing requirements for certain amino acids. D'Mello (1978) quoted the results of March and Biely (1972) for white leghorn cockerels fed graded lysine levels at two environmental temperatures, 20 and 31°C. D'Mello (1978) demonstrated that two distinct curves could be obtained when the responses were considered in relation to dietary concentration of lysine, which suggests a decrease in the efficiency of utilisation of lysine at 31°C. When the same results were plotted against daily intake of lysine, a single response curve was obtained, which indicated that the utilisation of lysine had not been impaired at the higher temperature, and that the daily intake of lysine required to promote a given amount of weight gain was similar at the two temperatures examined. This result may be due to the relatively low food intake of chicks maintained at 31.1°C, which necessitated higher dietary concentrations of lysine to compensate for a reduction in appetite (D'Mello, 1978).

c. Age and size of fish

Halver (1976a,b) indicated that the dietary concentrations of amino acids required by fish may drop as the fish grew larger. As yet no data appears to be available for the amino acid requirements of growing fish. Graber et al (1971b) determined the dietary methionine requirements of the chick at three stages of life. It was found that the dietary requirements during the 2nd, 5th and 8th weeks of life were 2.6, 2.2 and 2.1 g/kg.

Boomgaardt and Baker (1973b) examined the effect of age on the

lysine and sulphur amino acid requirements of the growing chick at two stages of growth: firstly, 14 to 28 days post-hatching and secondly, 42 to 56 days post-hatching. They found that the lysine requirement for maximal growth rate was constant (46.2 g/kg dietary protein) with increasing age. In contrast, the sulphur amino acid requirement was found to decrease from approximately 30.5 g/kg dietary protein during the third and fourth weeks to approximately 25.6 g/kg during the seventh and eighth weeks of the chick's life. The latter findings, however, are in direct conflict with those previously reported by Graber et al (1971b).

It is clear that the amino acid requirements, as g/kg diet or g/kg dietary protein, of animals decrease with advancing age. Whether these variations in amino acid requirements can be attributed merely to age or to differences in food intake needs to be investigated.

d. Nutritional factors

Various nutritional factors have been identified as influencing amino acid requirements of animals, including dietary protein level, energy concentration, vitamin B12 and the disproportionate amounts of dietary amino acids. These factors have been studied extensively with rats, chicks and pigs. Few efforts have been made to study the effect of these factors on the amino acid requirements of fish.

(i) Dietary protein level

The requirements of chicks (Almquist, 1952) and rats (Bressani and Mertz, 1958) for certain amino acids were found to be dependent upon the dietary protein level. These requirements (as g/kg diet) were found to decrease as the dietary protein concentration increased.

Almquist (1952) has shown that the lysine requirement of the chick decreased from 58 to 48 g/kg diet when the protein level increased from 100 to 400 g/kg diet. Similarly, the total sulphur amino acid requirement also decreased from 45 to 33 g/kg diet following an increased protein level. Bressani and Mertz (1958) reported similar observations with weanling rats. It was found that rats required about 67, 52 and 22 g of lysine/kg diet at protein concentrations of 80, 160 and 400 g/kg diet respectively.

(ii) Dietary energy concentrations

Dietary energy level is another factor that affects amino acid requirements of animals. Boomgaardt and Baker (1973a) studied the effect of three energy levels, 10.9, 12.6 and 14.2 MJ/kg diet, on the total sulphur amino acid requirements of the chick. Their findings on methionine and cystine requrements of chick have been considered in terms of dietary concentrations and daily intake by D'Mello (1978). Three distinct curves were obtained (Fig. 7) when the daily weight gain was plotted against the dietary levels of sulphur amino acids, implying a decrease in the efficiency of utilisation of methionine and cystine with increasing dietary energy concentrations. A single response curve (Fig. 8) was

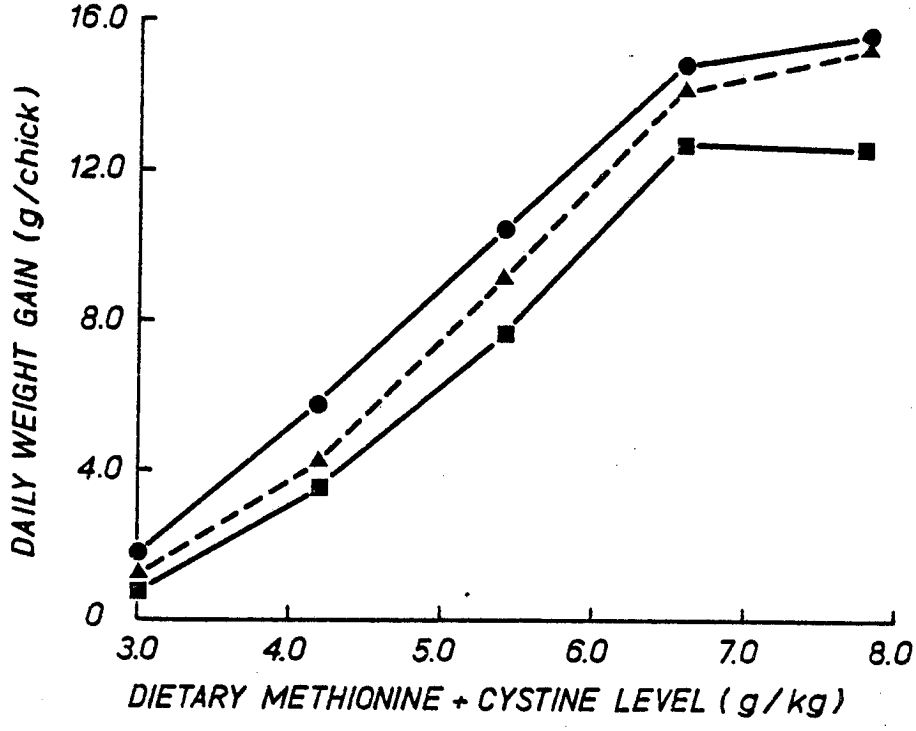


Fig. 7 Daily weight gain (g/chick) of young chicks in relation to dietary methionine + cystine (g/kg) and metabolisable energy concentrations. Energy levels (MJ/kg): (●) 10.9, (▲) 12.6, (■), 14.2. (After D'Mello, 1978)

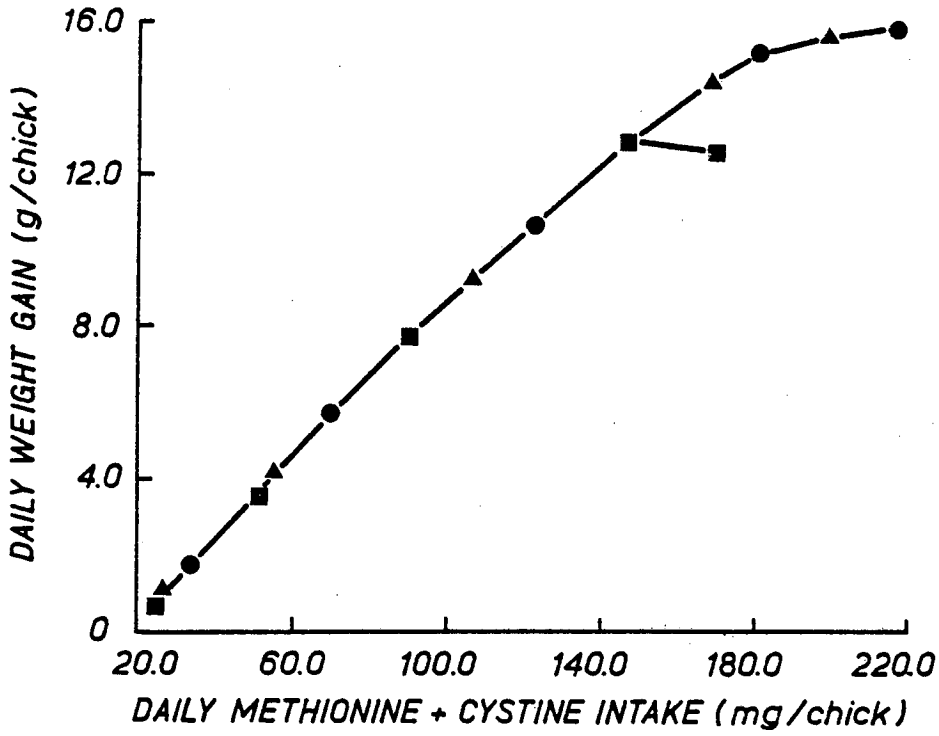


Fig. 8 Daily weight gain (g/chick) and daily methionine + cystine intake (mg/chick) at three dietary levels of metabolisable energy (MJ/kg): (●) 10.9, (▲) 12.6, (■) 14.2. (After D'Mello, 1978)

obtained by plotting weight gain against the intake of methionine and cystine. From these results it appears that dietary energy concentrations influence the voluntary food intake and not the utilisation of sulphur amino acids.

(iii) Vitamin levels

As has been indicated earlier, the tryptophan requirement of certain animals is dependent upon the niacin concentration (Krehl et al, 1945; Almquist, 1959; Harper, 1964), although such a relationship has not so far been proved with fish species (Poston and DiLorenzo, 1973; Wilson et al, 1978). Vitamin B12, choline and betaine have also been reported to affect the requirement of chicks for methionine.

Recently, studies with chicks have shown that their sulphur amino acid requirement is dependent upon the level of vitamin B12 (Looi and Renner, 1974 in D'Mello, 1978). It was indicated that the chick required less methionine and cystine when adequate concentrations of vitamin B12 were included in the diet. However, D'Mello (1978) demonstrated that the utilisation of the sulphur amino acids was not affected by vitamin B12 deficiency, and that the daily need for a given growth rate was similar at two different levels of vitamin B12.

Choline and betaine have been shown to influence the methionine requirement of chicks (Pesti et al, 1978). This arises from the function of these compounds as methyl donors. Cystine or sulphate

supplements failed to promote growth or efficiency of food conversion in chicks fed diets deficient in methionine. When the same diets were supplemented with choline or betaine, indistinguishable growth performance from that achieved with methionine supplements was obtained. However, Pesti et al (1979) concluded that there is no reason to supplement the chick diet with methionine if there are sufficient methyl donors (choline and betaine) present in the diet. It is clear that the amino acid requirements, as g/kg diet, of terrestrial vertebrates are influenced by vitamin concentrations. Whether the amino acid requirements of fish are influenced by the levels of dietary vitamins in the same manner as chicks needs detailed study. It would also be of interest to speculate on the effect of dietary concentrations of vitamins on amino acid utilisation by fish.

(iv) Disproportionate amounts of dietary amino acids

The adverse effects from ingestion of diets containing disproportionate amounts of amino acids have been observed in experiments conducted on rats (Harper, 1958; Sauberlich, 1961; Harper et al, 1970), chicks (D'Mello and Lewis, 1970a,b,c) and fish (Chance et al, 1964; Halver, 1976a,b; Robinson et al, 1981). These adverse effects ranged from moderate depression in food intake and growth to the development of pathological lesions resulting in high mortality. Three terms, viz., toxicity, antagonism and imbalance, have been used by different authors in different ways to report the adverse effects due to ingestion of diets containing disproportionate amounts of amino acids (Harper et al 1970).

Toxicity is the adverse effect resulting from excessive intake of individual amino acids which is manifest in pathological lesions, growth retardation and depression of food intake, and which can lead to mortality. On the other hand, any modification of the dietary amino acid profile, a proportional decrease or increase in all amino acids excepted, is referred to as amino acid imbalance. Amino acid antagonism is defined as the competition of structural analogues of amino acids with natural ones, which may result in inhibition of protein synthesis, blocking of an enzymatic reaction or competition for transport sites (Harper, 1970). The term "amino acid interaction" has been used frequently in chick studies, however, and is considered to be equivalent in meaning to amino acid antagonism (D'Mello, 1970a,b,c).

The interdependence in the amino acid requirements of the chick has been studied and defined in quantitative terms by D'Mello and Lewis (1970a,b,c). They demonstrated that arginine requirements increased linearly with increasing levels of excess dietary lysine concentrations (D'Mello and Lewis 1970a). A similar dependence of isoleucine and valine upon the dietary level of leucine (D'Mello and Lewis 1970b), and tryptophan requirements upon the dietary level of threonine, was also reported (D'Mello and Lewis, 1970c).

Studies with fish have shown that an imbalance of isoleucine with respect to leucine could be as harmful to growth as an imbalance of leucine with respect to isoleucine (Chance et al, 1964; NAS/NRC, 1973; Halver, 1976a,b). Chance et al (1964)

demonstrated the retardation of growth in chinook salmon fingerlings fed increasing levels of isoleucine in the presence of a constant concentration of leucine.

A series of experiments was conducted to evaluate the effects of excessive levels of dietary lysine and arginine on growth responses and efficiency of food conversion of channel catfish (Robinson et al, 1981). It was found that at adequate or marginal dietary levels (10.0 and 7.5 g/kg) of arginine, excessive dietary concentrations of lysine ranging from 12.3 - 49.2 g/kg had no significant effect on the performance of channel catfish. This result indicated that the levels of excess lysine in the diet did not induce an arginine deficiency, and therefore that arginine requirements were independent of lysine concentrations in the diet. These findings are in direct contrast with those reported by D'Mello and Lewis (1970a) who recorded linear increases in arginine requirements of chicks with increased levels of excess lysine in the diet.

In the same study, Robinson et al (1981) demonstrated the absence of antagonism in channel catfish when fed excessive levels of dietary arginine at the marginal level (75% of the requirement) of lysine. This report also provided no evidence of an arginine-lysine interaction in channel catfish.

On the basis of the work by Chance et al (1964), Halver (1976a,b) indicated that an inhibition of growth and diet performance was observed either when isoleucine was threefold in

excess of leucine, or when a threefold level of leucine was fed with the isoleucine held at the requirement level. He also indicated that a high intake of valine inhibited growth in salmonoids. High levels of valine did not inhibit growth, however,but when these were included in the diet at a level over 30 g/kg diet, low growth rate and efficiency of food conversion were observed (NAS/NRC, 1973).

F. Objectives of the Current Studies

Quantitative data on protein and amino acid requirements of warm water fish is relatively scarce. The only available findings on the amino acid requirements of carp are those published by Nose (1978) using test diets in which amino acids formed the sole source of protein. That study was hampered by a lack of data on food intake and efficiency of food conversion. These two criteria are the most important in studies involving estimation of the nutrient requirements of animals. In addition, Nose's data concerning the performance of fish on different concentrations of the amino acid under study was not analysed statistically. Minimum amino acid requirements for optimum performance cannot, therefore, be safely extrapolated.

It is generally recognised that various factors affect the dietary protein requirements of fish. The indispensable amino acids are the most important constituents of dietary protein. Experience with different animal species has shown that their amino acid requirements as dietary concentrations are influenced by

environmental, physiological and nutritional factors. However, these animals are mostly similar in their amino acid utilisations even when various factors are involved, if amino acid intake is taken as a measure of the requirement. When these requirements are viewed in terms of daily intake, similarities in amino acid utilisation by terrestrial vertebrates have emerged (D'Mello, 1978). Whether the various species of fish are similar to endothermic animals in utilising amino acids needs to be investigated.

The first objective of the present study is to determine the protein and amino acid requirements, in terms of dietary concentrations and intake, of mirror carp, utilising combinations of natural proteins relatively deficient in the amino acid under test. The second objective is to find out whether the requirements for dietary indispensable amino acids can be lowered by using a combination of natural protein sources rather than by using purified amino acid test diets. The third objective is to discover if there is any change in these requirements with age and water temperature when such requirements are viewed as a function of dietary concentrations and intake. The fourth objective is to investigate whether the chemical composition of fish tissue is affected by the level of protein or amino acid. Finally, this study sets out to compare the amino acid requirement of carp with that of other species of fish and several terrestrial vertebrates.

II - Materials and Methods

A. Experimental Fish

During the course of the current studies, fingerling mirror carp (Cyprinus carpio L.) were obtained from three sources. For experiments 2,3,4,6,7 and 11, carp were obtained from Humberside Fisheries, Yorkshire, England. Experiments 1, 5, 8, 10, 12 and 13 were conducted on fish obtained from the Severn Trent Water Authority Calverton Fish Farm, Nottingham, England. Fish used in experiment 9 were obtained from Newhay Fisheries, Yorkshire, England. The size of fish selected for experimental purposes ranged between 7 and 12 cm. Although attempts were made to use fish of approximately the same size (9.19 cm) in all experiments, this factor varied ($\pm$ 2.5 cm) from one experiment to another. Due to the difficulties associated with obtaining fish in the winter season, the fish in experiments 1, 3 and 6 were used again in experiments 8, 4 and 11 respectively.

B. Fish Transport and Culture

On all occasions, fish were transported in sealed black polythene bags (100-130 fish/bag) containing a large volume of oxygen over a small volume of water, as suggested by Tylor and Solmon (1979). Damage was avoided by placing each bag inside a rigid container during transport. In the laboratory, the fish were divided into groups according to the number of experimental treatments, and maintained in glass aquaria (25 h x 25 w x 70

length cm). Each tank was covered with a polyvinyl chloride mesh and a black polythene sheet to ensure similarity in lighting. In experiments 3 and 4, the tanks were uniformly aerated by air pumps (Hyflo) and equipped with heaters (Hi-duty, Medcalf Bros. Ltd) and thermostats (Super steady stat, Interpet) to maintain the desired temperature ($\pm$ 1°C). In the remaining experiments (1, 2 and 5-13), aeration for each tank was provided by means of an air compressor (B.V.C. Ltd). The desired water temperature (20 or 25 $\pm$ 1°C) in these experiments was regulated in a temperature controlled room using an air conditioner (Searle-Bush) incorporating a thermostat.

C. Acclimation and Feeding

Prior to the start of each experiment, all fish underwent conditioning over a period of 2-4 weeks. During this period the daily lighting routine was 9-10 h, except Sunday when it was 3-4 h. The tanks were cleaned every 48 h without removing the fish. This cleaning ensured that algae growth and faecal accumulation were kept to a minimum. Once placed in this artificial habitat, the fish usually began to search for food within a period of 3-5 days. They were initially maintained on a diet of paste-like consistency which was similar in composition and physical characteristics to the experimental diets.

During the acclimation and experimental periods, the fish were fed by one individual on a rigid schedule 3 times a day (09.30, 13.30 and 17.00), except Sunday when they were fed once at noon. On each occasion, fish were fed to satiation in order to achieve

maximum growth and protein utilisation (Gerking, 1952). Since exact food intake was an important measurement in this study, and leaching of nutrients from the diet was possible, experimental fish were trained to pick up food just below (5-10 cm) the water surface. Feeding was stopped as soon as any food reached the bottom of the tank and the fish ceased to show any interest. Rejected food was siphoned off, air dried and stored at -20°C until analysed for dry matter (DM).

The tanks were inspected twice daily to remove dead fish and to ensure a continuous air flow. At the end of the acclimation period, the fish were expected to feed actively on the test diets. Undesirable fish were eliminated on the basis of size, weight and deformities, and the remaining fish were randomly distributed in equal numbers to the experimental tanks which, were assigned at random.

D. Marking and Weighing

In each experiment, prior to marking, fish were anaesthetised using ethyl m-aminobenzoate sulphonate (MS 222, Sandoze Ltd) or ethyl p-aminobenzoate (Benzocain, BDH Chemicals Ltd.) in a concentration of 20-25 mg/l (Laird and Oswald, 1975). Each fish was captured, rolled on a slightly damp tissue for 10 seconds to remove surface moisture, and marked by using a jet inoculator to inject a spot of dye (Alcian blue) under the skin. Up to 20 fish could be marked and distinguished by colour spots injected at different sites on the left or right side of the body (Fig. 9).

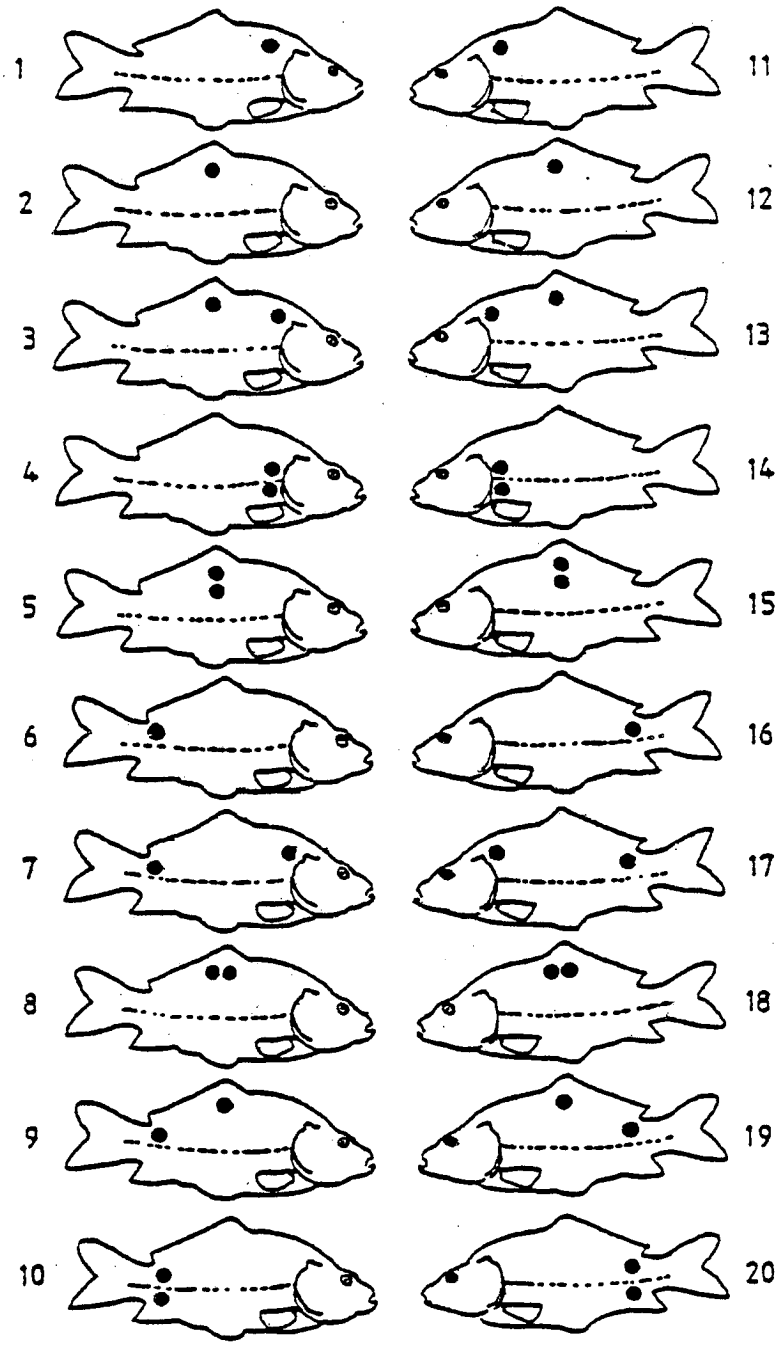
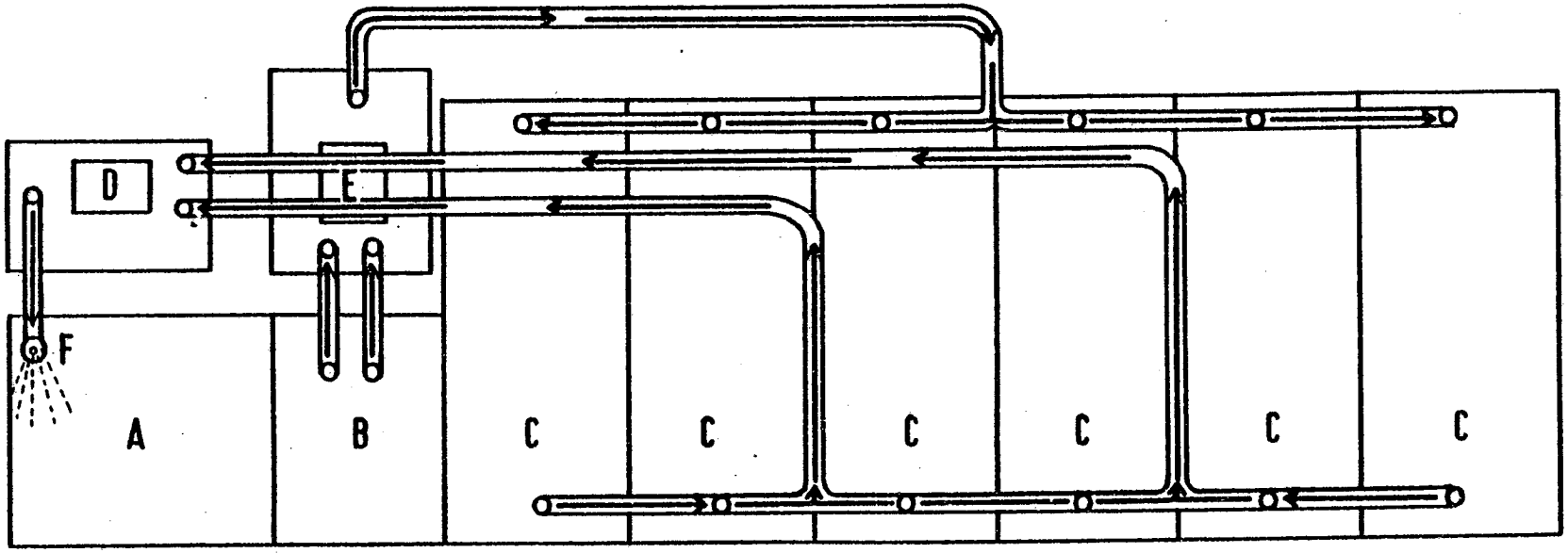


Fig. 9 Twenty marked fish injected with dye at different sites on each side of the body.

SCHEMATIC DIAGRAM OF WATER RECYCLING SYSTEM



A. Biological Filter
B. Cleaning Oven
C. Aquaria
D. Power Filter
E. Power Filter
F. Water Fall

Individual wet weight was determined by placing the marked fish into a known weight of water (Lagler, 1978). Weight was recorded to the nearest 0.01 g by using an electronic balance (Sartorius) at the beginning, in the middle and at the end of each experiment. On each occasion, fish were starved for about 36 h before weighing in order to allow evacuation of the gastrointestinal tract. The duration of the experiments was 6 weeks except for experiments 3 and 4, which lasted for 8 weeks.

E. Water Supply and Quality

Mains water in the Department of Forestry and Natural Resources was used. Water quality varied among some groups of experiments for several reasons.

Experiment 3 was conducted with two recycled water systems. Each system consisted of 6 aquaria, one biological filter unit and two power filters to recirculate the water through plastic pipes. Each biological filter (gravel) unit consisted of one tank divided into two parts by a thin plastic wall, which was perforated at the lower end to permit water passage from one part to another. (See facing page).

The capacity of each water system was about 175 l, and of this water about 86% was available for the experimental fish. The turnover rate of the water-fall at the biological filter was adjusted to 300 l/h. The main purpose of the water-fall was to increase the amount of dissolved oxygen, which is required by the aerobic bacteria for more efficient denitrification (Spotte, 1970).

Similar water flow of 50 l/h from the cleaning tank to each aquarium and from the latter to the gravel bed was achieved by using metallic restrainers.

Although the main function of the power filters was to pump and recirculate water throughout the system, they also served as mechanical and chemical filters, using polymer filter wool and charcoal respectively to trap faecal materials. Every three days the power filters and charcoal were rinsed with water and the floss replaced. At the same time, 25 l of water were gradually siphoned from the clearing tank and replaced by fresh water. In order to avoid overflowing or totally emptying the tanks, each system was equipped with automatic level switching.

The growth of carp measured in the middle of experiment 3 was slightly higher than expected, and contamination from the high lysine treatments to the lower ones via the water circulation system was considered to be a probable reason at that time. At the end of the experiment, immediately after the last feeding, water samples were taken from the experimental tanks for amino acid analysis.

Although water analysis proved that detectable levels of amino acids were present in the water, it could not be shown that there was a transfer of nutrients between treatments via the circulation system. Since this is possible, however, and because carp are able to absorb nutrients through their skin (Oosten, 1957; Ghittino, 1972), a different water supply system was used in the remaining

experiments (1, 2 and 4-10). Accordingly, about 90% of the water from each tank was replaced with fresh water every 48 h during the course of each of these experiments. Such a procedure required a large amount (600-700 1) of dechlorinated water, warmed to experimental temperature every 48 h. Eight plastic 75 1 containers containing charcoal were used to this purpose.

Due to difficulties in obtaining large amounts of water supplied through PVC pipes, tap water was used. To avoid a high copper level (1.56 mg/1) which would be toxic to fish (Alabaster, 1979)[1], a water deioniser (Permutit MK 8) was used in experiments 1, 5 and 7-13. In these experiments, charcoal was removed from the storage containers.

F. Experimental Diets

The basal diets employed in all experiments were prepared from various intact protein sources. Amino acid, crude protein and ME contents of these ingredients are summarised in Table 15. The values for protein presented in all related tables in the current investigation should be treated as a crude protein (N x 6.25) estimate. The composition of the various basal diets used in the present study are shown in Table 16. An average crude protein level of approximately 370 g/kg diet (Ogino and Saito, 1970) was maintained in all diets except for those experiments (1 and 2) which involved protein requirement studies. On the basis of findings reported by Nose (1978), all the basal diets employed were designed to be deficient in the amino acid under test, but

[1] Dr. J. S. Alabaster (personal communication)

TABLE - 15

Amino acid composition (g/kg DM) of ingredients used.

Amino acid	Whole wheat	Maize gluten	Fish meal	Casein	Bacterial protein	Hydrocarbon -grown Yeast	Gelatin	Zein	Soyabean	Wheat Gluten
Aspartic acid	5.93	45.90	59.34	52.64	-	58.74	55.90	-	-	26.23
Threonine	3.19	24.97	32.57	37.74	33.87	26.40	17.60	25.63	19.26	20.06
Serine	5.47	38.42	40.60	55.72	-	27.78	29.40	-	26.44	37.41
Glutamic acid	31.36	178.32	100.11	220.60	-	94.38	100.00	247.04	-	342.33
Glycine	4.79	18.76	69.84	20.41	37.23	27.04	242.10	10.21	20.79	26.44
Alanine	4.45	66.90	44.26	31.89	-	38.07	90.20	-	-	20.79
Valine	5.82	32.88	43.17	76.59	38.53	31.71	23.50	37.86	22.09	30.20
Cystine	2.28	11.98	10.93	2.87	4.76	5.30	1.00	8.51	6.09	18.39
Methionine	1.25	18.42	18.58	27.04	17.32	8.80	7.80	18.50	6.09	12.43
Isoleucine	4.10	29.72	31.48	57.16	33.44	25.87	13.70	38.60	20.35	27.69
Leucine	7.64	132.44	52.24	102.63	57.47	41.89	28.40	202.27	35.70	55.38
Tyrosine	3.99	39.33	26.99	60.03	24.24	23.86	4.90	48.28	18.28	28.74
Phenylalanine	4.45	45.99	32.90	54.51	28.25	23.54	7.80	68.17	22.85	38.25
Lysine	3.42	11.98	39.78	90.38	44.70	43.16	31.40	0.74	28.62	13.48
Histidine	2.85	15.48	13.00	32.66	13.53	11.45	5.90	11.38	12.84	16.51
Arginine	5.25	23.84	46.34	39.95	35.17	29.37	66.60	14.46	33.74	29.78
Tryptophan	1.37	4.97	7.65	10.48	7.58	7.95	0.00	2.02	6.53	7.52
Protein[a]	125.46	678.00	674.33	1026.30	757.60	604.45	1253.12	893.30	467.97	835.98
ME (MJ/kg)	10.69[b]	18.57[c]	17.30[c]	17.33[b]	10.92[d]	13.15[d]	9.88[e]	9.88[f]	19.48[d]	9.88[f]

[a] Crude protein (N x 6.25)
[b] NAS/NRC (1977a)
[c] Smith et al (1980)
[d] D'Mello and Acamovic (1976)
[e] NAS/NRC (1977b)
[e] assumed as in gelatin

TABLE - 16

Composition (g/kg) of the basal diets used in all experiments.

Experiment No:	1					2		3,4,5	6,7,8	9	10	11	12	13
Diet: / Ingredient	P1[a]	P2[a]	P3[a]	P4[a]	P5[a]	P1[a]	P2[a]	LO	MO	MOCO	TO	TO	HO	ThO
Whole Wheat	310.0	256.0	254.0	247.0	252.0	250.0	245.0	245.0	120.0	-	200.0	120.0	97.0	370.0
Fish Meal	80.0	80.0	80.0	80.0	80.0	100.0	80.0	155.0	-	-	-	-	104.5	-
Maize Gluten Meal	-	-	-	-	-	-	-	50.0	-	-	50.0	-	-	-
Zein	-	-	-	-	-	-	-	90.0	50.0	-	90.0	75.0	69.5	-
Hydrocarbon-grown Yeast	60.0	60.0	60.0	60.0	60.0	70.0	50.0	10.0	42.0	64.0	32.0	45.0	22.5	21.0
Wheat Gluten	-	-	-	-	-	25.0	180.0	180.0	-	-	50.0	-	-	94.0
Gelatin	-	-	-	-	-	-	-	-	165.0	200.0	150.0	165.0	189.8	196.0
Casein	15.0	30.0	42.0	60.0	75.0	-	-	-	90.0	91.0	-	35.0	-	24.0
Soyabean	10.0	75.0	140.0	180.0	230.0	-	-	-	90.0	61.0	-	124.0	-	-
Bacterial Protein	20.0	40.0	56.0	80.0	100.0	55.0	40.0	-	-	32.0	40.0	-	-	-
Corn Starch	377.0	331.0	240.0	165.0	75.0	328.5	246.6	135.4	303.0	419.0	246.0	294.0	365.5	150.0
α-Cellulose	20.0	20.0	20.0	20.0	20.0	40.0	40.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin Mix	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
Mineral Mix	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Amino acid Mix[b]	-	-	-	-	-	23.5[b1]	10.4[b2]	6.6[b7]	8.0[b4]	5.0[b5]	14.0[b6]	14.0[b7]	15.0[b8]	17.0[b9]
Cod Liver Oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Corn Oil	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0

[a] protein levels

[b] amino acid content (g/kg diet):

[b1] L-Thr (6), L-val (2), DL-Met (5), L-Phe (4), L-His-HCl (1), Free L-Arg (5) and L-Try (0.5 g/kg)

[b2] L-Thr (4), DL-Met (4) and free L-Arg (2.3 g/kg)

[b3] L-Thr (2.3) and DL-Met (4.3)

[b4] L-Thr (5), Phe (2) and L-Try (1 g/kg)

[b5] L-Phe (4.5) and L-Try (5 g/kg)

[b6] L-Thr (5), DL-Met (3), L-Phe (1) and L-Lys-HCl (5 g/kg)

[b7] L-Thr (5), DL-Met (5), L-Phe (2) and L-His-HCl (2 g/kg)

[b8] L-Thr (5), L-Val (2), DL-Met (4.5), L-Phe (2)and L-Try (1.5 g/kg)

[b9] L-Val (3), DL-Met (5), L-Phe (5), L-His-HCl (3) and L-Try (1 g/kg

contained all other amino acids at a level required for optimal growth (after Nose, 1978). The various supplements of amino acids were effected using crystalline amino acids. It was assumed that all diets used in an experiment (except the diets of experiment 1 and both basal diets of experiment 2) were isonitrogenous, and that the amount of protein (calculated after Florkin and Stotz, 1963) added in the form of the treatment amino acids was negligible. The amino acid contents of the various basal diets are shown in Table 17. Vitamin and mineral mixtures (See Appendix A, Tables A1-A3) were included in the basal diets to ensure adequate intake of these essential nutrients. The approximate analyses of the experimental diets of each experiment are shown in Appendix B, Tables B1-B7.

1. Protein requirement studies

In experiment 1, five experimental diets (1P1-1P5) were designed to contain 5 dietary protein concentrations, 181, 243, 282, 334, and 389 g/kg DM, achieved by gradually increasing the level of casein from 15 to 75 g/kg diet, soyabean from 10 to 230 and bacterial protein from 20 to 100, and at the same time by decreasing the level of corn starch from 377 to 75 g/kg diet (Table 16). The other dietary ingredients were kept constant, except for whole wheat which was slightly higher in the first protein diet. Since the aim of this experiment was to investigate the utilisation, at 20°C, of different dietary protein levels as provided by the protein sources used, no attempt was made to supplement these diets with purified crystalline amino acids or to adjust the amino acid pattern to approximate that recommended by

TABLE - 17

Amino acid composition[a] (g/kg DM) of the basal diets.

Experiment No:	1					2		3	4	5	6	7	8	9	10	11	12	13
Diet: / Amino acid	P1	P2	P3	P4	P5	2P1LO	2P2LO	3LO	4LO	5LO	6MO	7MO	8MO	9MOCO	10TO	11TO	12HO	13ThO
Aspartic acid	-	-	-	-	-	16.73	18.37	24.96	25.82	25.64	28.56	28.35	28.46	28.65	-	-	22.23	19.57
Threonine	6.10	8.20	10.30	12.40	14.40	13.70	13.46	17.45	18.06	17.93	16.29	16.17	16.24	10.72	14.50	14.30	12.49	8.41
Serine	-	-	-	-	-	9.51	13.61	23.05	23.85	23.68	18.44	18.31	18.38	16.78	-	-	11.99	14.78
Glutamic acid	-	-	-	-	-	4.05	87.70	156.08	161.49	160.32	76.12	75.55	75.85	60.02	-	-	53.06	80.86
Glycine	-	-	-	-	-	11.88	14.30	18.65	19.30	19.16	48.66	48.30	48.50	53.08	-	-	47.61	51.23
Alanine	-	-	-	-	-	12.30	13.29	25.81	26.70	26.51	29.02	28.80	28.92	25.99	-	-	27.70	23.78
Valine	8.50	11.30	14.00	17.00	19.80	12.49	13.96	20.28	20.99	20.83	16.21	16.09	16.15	15.49	12.60	12.90	13.76	16.03
Cystine	1.70	2.30	2.70	3.20	3.50	2.81	5.39	5.54	5.73	5.69	2.43	2.42	2.43	-	3.00	1.80	2.22	4.03
Methionine	2.90	3.90	4.90	6.00	6.90	9.47	8.29	14.65	15.15	15.04	6.10	6.05	6.07	4.49	8.00	9.40	9.09	9.82
Isoleucine	6.50	8.90	11.20	13.70	16.00	8.36	11.77	16.44	17.01	16.89	11.87	11.78	11.83	11.51	10.30	10.20	8.71	8.95
Leucine	11.10	15.30	19.40	23.60	27.70	14.96	21.71	45.81	47.39	47.05	29.93	29.71	29.83	21.87	34.70	29.10	24.95	18.17
Tyrosine	5.90	8.10	10.30	12.40	14.50	9.12	11.80	17.99	18.61	18.48	14.45	14.34	14.40	11.98	10.70	10.10	9.62	9.25
Phenylalanine	6.30	8.80	11.20	13.60	15.80	12.23	15.07	23.20	24.00	23.83	18.09	17.95	18.03	19.41	16.10	15.90	16.50	19.14
Lysine	8.50	12.30	15.50	18.90	22.30	11.75	12.59	12.11	12.53	12.44	17.62	17.49	17.56	22.19	15.40	17.40	13.37	12.57
Histidine	3.20	4.50	5.80	7.10	8.40	5.12	8.86	9.13	9.45	9.38	7.07	7.02	7.04	6.62	5.10	7.40	5.20	8.61
Arginine[b]	8.00	11.00	13.90	16.60	19.40	16.23	16.54	18.09	18.71	18.58	24.25	24.07	24.16	26.19	17.50	20.10	21.97	23.59
Tryptophan[b]	1.70	2.50	3.00	3.70	4.20	2.20	2.90	3.20	3.20	3.20	1.90	1.90	1.90	2.00	1.50	1.50	2.60	2.50

[a] determined values
[b] calculated values

Nose (1978). The amino acid composition of these diets is listed in Table 17.

2. Amino acid requirement studies

In all amino acid studies, the graded supplementation technique has been employed since D'Mello (1982) demonstrated that this technique is in no way inferior to the diet dilution technique in the assessment of the amino acid requirements of non-ruminant animals.

The aim of experiment 2 was to determine the lysine requirement, at 20°C, of carp fed on diets containing two protein concentrations. The two basal protein diets (2P1L0 and 2P2L0) employed in this experiment were designed to furnish about 218 and 300 g protein/kg DM respectively by using similar dietary protein sources, but in varying amounts (Table 16). The lysine concentrations in these basal diets were adjusted to be 11.8 and 12.6 g/kg DM respectively (see Table 17). In order to obtain six experimental diets in factorial arrangement, each basal protein diet was supplemented with 2 and 4 g L-lysine/kg diet (presented as 2P1L1 and 2P1L2 for the lower protein level and 2P2L1 and 2P2L2 for the higher protein level).

Experiments 3, 4 and 5 were planned to assess the lysine requirement of carp using a recycled water system at 20°C, a non-recycled water system at 20°C, and a non-recycled water system at 25°C respectively. The basal diets, 3L0, 4L0 and 5L0

respectively of these experiments were identical in terms of ingredients (Table 16). On analysis, the lysine levels in the basal diets were found to be 12.1, 12.5 and 12.4 g/kg DM respectively (Table 17).

Five supplemented diets (L1-L5) were formulated from the basal diets (L0) of experiments 3 (3L0) and 5 (5L0) by adding graded concentrations of L-lysine.HCl in increments of 2 g/kg diet. From the basal diet of experiment 4 (4L0), only three supplemental diets (L1-L4) were derived using the same incremental addition of dietary lysine as in experiments 3 and 5.

The objective of experiments 6 and 8 was to determine the dietary methionine requirements at 20 and 25°C respectively, whereas experiment 7 was planned to investigate the utilisation of the L-isomer of methionine at 20°C. The ingredient composition of the basal diet used in these experiments was identical (see Table 16). Each basal diet was designed to contain 6.1 g methionine/kg DM and 2.4 g cystine/kg DM (Table 17) by adjusting the concentration of protein sources used. From each basal diet (M0), four supplemented diets (M1 - M4) were formulated by the addition of synthetic DL-methionine (in experiments 6 and 8), and L-methionone (experiment 7) in increments of 1.5 g/kg.

Experiment 9 was designed to investigate the sparing action of cystine on methionine requirements in factorial combination at

25° C. One basal diet (9M0C0) containing 4.5 g methionine and 1.1 g cystine/kg DM (Table 17) was employed (Table 16). From this diet, two methionine supplemented diets (9M1C0 and 9M2C0) were prepared by the addition of DL-methionine in increments of 3.0 g/kg. From the original basal diet (9M0C0) and from each of the methionine supplemented diets (9M1C0 and 9M2C0), three cystine supplemented diets were prepared by the addition of L-cystine in increments of 1.0 g/kg, and presented as M0C1, M0C2, M0C3, M1C1, M1C2, M1C3, M2C1, M2C2, M2C3 respectively.

Experiments 10 and 11 were designed to determine the tryptophan requirement of carp at 20°C. The basal diets of experiment 10 (10T0) and 11 (11T0) were formulated from ingredients listed in Table 16, and adjusted to contain 1.5 g tryptophan/kg DM (Table 17). From each basal diet, three supplemented diets (T1, T2 and T3) were formulated by the addition of crystalline L-tryptophan in increments of 0.5 g/kg.

Experiments 12 and 13 were planned to estimate the requirements of carp for dietary histidine and threonine respectively at 20°C. The histidine concentration in the basal diet (12H0) of experiment 12 (Table 16) was adjusted to be 5.2 g/kg DM as indicated in Table 17. Four experimental diets (H1-H4) were prepared by the addition of L-histidine.HCl to the basal diet in increments of 0.5 g/kg. In experiment 13, the basal diet (Th0) was formulated from ingredients listed in Table 16 to contain 7.3 g threonine/kg DM. The amino acid composition of this basal diet is shown in Table 17. From this diet, four supplemented diets (Th1-Th4) were prepared by the

addition of L-threonine in increments of 1.5 g/kg.

G. Diet Preparation

The basal diet, excluding the oils, of each experiment was ground into a homogenous powder, then mixed thoroughly for 15 min using a Hobart mixer (AE 200) and divided into equal portions according to the intended number of treatments. The incremental amount of the crystalline amino acid was added to each portion to formulate the supplemented diets. At this stage each experimental diet was mixed thoroughly for 15 min and stored until used. Moist food in excess of immediate requirements was prepared for each treatment group every two weeks by thoroughly mixing the dry ingredients with oil and then adding water (at 80°C) until a stiff dough resulted (Halver, 1957b). This was then passed through a domestic mincer, and the resulting strings of material collected and divided into 28 equal portions and stored in the deep freeze (-20°C) until used. Each portion represented the daily ration to be offered for each replicated treatment. Prior to feeding, each portion was thawed for 15-20 minutes.

H. Sampling Procedure and Chemical Analysis

1. Ingredients and diets

Representative samples weighing about 50 g were taken from various test diets used in each experiment for proximate analysis. DM was estimated by oven drying at 100°C for 24 h. The GE of each test diet excluding the oil was determined in a bomb

bomb calorimeter. The GE of fat was assumed to be 39.4 MJ/kg calculated as an average of four kinds of oil (HCP, 1947). The protein content was determined by the Kjeldahl procedure. Crude fat was extracted using methanol chloroform, and crude ash was assessed by burning in a muffle furnace at 550°C for 12 h (AOAC, 1970).

Samples of ingredients and basal diets were analysed for amino acids by ion-exchange chromatography. Details of methods used are given by D'Mello (1972; 1973). All diet composition data in Table 17, Tables A1-A3 (Appendix A) and Tables B1-B7 (Appendix B) refer to the complete diets, including the oil additions.

2. Fish

At the start of each experiment, samples of 20-25 fish were randomly selected from the stock and killed by an overdose (50-70 mg/l) of anaesthetic (Laird and Oswald, 1975). In order to obtain approximately the same degree of hydration, surface moisture was removed as described earlier in Section II-D. The dead fish were then stored in polythene bags at -20°C for subsequent analysis. At the termination of the experiment, an equal number of samples were taken randomly from each group, killed by anaesthetic, their hydration standardised, and then stored at -20°C. The frozen fish of each group were cut into small pieces, mixed and blended thoroughly. The tissues were then divided into two portions. One was used for duplicate DM determination while the other portion was freeze-dried, ground into a homogenous powder and analysed for protein, fat, GE and ash as indicated in Section II-H-1.

3. Water

The aquarium water was monitored intermittently for ammonia and dissolved oxygen. The concentration of ammonia in the water was determined using a pH meter with an ammonia probe (7030 Electronic Instruments Ltd.). In any determination, the ammonia level in the aquarium water did not exceed 0.25 mg/l. The dissolved oxygen concentration in the same water was estimated by the developed Winkler (1888, in Mackereth et al,1978) method (Direct reading, HACH DR-EL, Engineer's laboratory) and did not fall below 40% saturation. However, the highest ammonia levels and lowest dissolved oxygen concentrations were recorded in samples taken from aquaria in which the fish exhibited a high growth rate and were receiving an adequate diet.

The tap water supplied by both plastic and copper systems as well as the water from fish tanks was sampled and analysed for copper by the Water Supply Service of Lothian Regional Council. The concentration of copper in the tap water, plastic system and copper system was found to be 0.05, 0.15 and <0.05 mg/l respectively. At approximately the same time, similar samples were taken and then analysed for copper concentration by the Spectrochemistry Department, School of Agriculture. Results of this analysis have shown that the levels of copper were 0.07, 0.10 and 0.04 mg/l in the tap water, plastic system and copper system respectively.

I. Criteria for Assessing Requirements

1. Live weight gain

The measurement of live weight gain of carp was considered to be the main criterion of the adequacy of test diets offered. This selection was based on the assumption that there is a strong correlation between protein retention and weight gain in the growing animal. The average daily individual weight gain was recorded for the two replicate groups and expressed as g/fish/d.

2. Dry matter, nitrogen, gross energy and amino acid intake

The average daily individual DM intake was determined at the end of each experiment and expressed as g/fish/d. The protein, GE and amino acid intake were computed from the DM intake and from its gross content of these constituents. The protein and amino acid intake were expressed as mg/fish/d, whereas the GE intake was expressed as kJ/fish/d.

3. Efficiency of food conversion

The efficiency of food conversion was estimated as g weight gain per g DM ingested.

4. Protein, fat, gross energy and ash deposition

The average protein, fat, GE and ash depositions in fish tissue were calculated from the difference between the initial and the terminal samples, then divided by the number of experimental days.

Carcass deposition of protein, fat and ash was expressed as mg/fish/d. Carcass deposition of gross energy was expressed as kJ/fish/d.

J. Diseases and Treatments

During the first week of acclimation in each experiment, fish were treated either with potassium permanganate ($K\ Mn\ O_4$) solution (0.1 g/l) for 60-90 min, or with sodium chloride (NaCl) solution (10 g/l) for 20 min (Huet, 1974) in order to control fungal diseases. The sudden change of temperature from the pond to the fish tank may have been responsible for a serious outbreak of disease among the experimental fish (Experiments 5 and 8), the symptoms of which closely resembled those described by Snieszko and Axelrod (1971) for Columnaris (Flexibacter columnaris). This condition was treated by lowering the temperature to 15°C (Snieszko and Axelrod, 1971). For the above reason, room temperature was also adjusted to approximate the ambient temperature recorded a day prior to obtaining the fish. A gradual increase in room temperature up to the desirable limit was made during the acclimation period.

K. Experimental Design

Experiments 1, 3-7 and 9-13 were (simple) randomised block designs involving 4-6 treatments of the amino acid under investigation. Experiment 2 involved 2 levels of protein and 3 concentrations of lysine, whereas experiment 8 involved 3 levels of

methionine and 4 concentrations of cystine. Experiments 2 and 8 were therefore both straightforward, factorial designs. In all experiments, two replicates for each treatment were made. The treatments were randomised within each replicate group, to offset effects of size variation and environmental factors within the room housing the fish.

L. Statistical Analysis

All the data obtained from each experiment was analysed using the Rothamsted Experimental Statistical Program, Genstat. The results (mean of two replicates) of each experiment have been illustrated in tables which also show the standard error of the mean and degrees of freedom. Mean differences ($p<0.05$) were determined using t-test (Snedecor and Cochran, 1978) as follows:

$$t = \frac{M2-M1}{SEM\sqrt{2}}$$

In experiment 1, regression analysis was used to calculate the relationship between dietary protein concentration and daily weight gain, and daily protein intake and protein deposition in carp. In all experiments, protein or amino acid requirements were estimated from the growth responses to both dietary protein, or amino acid, concentration (g/kg) and daily intake (mg/fish).

III. EXPERIMENTS AND RESULTS

All the fish offered adequate diets soon became accustomed to accepting them and feeding actively during the course of each experiment. In contrast, fish given diets containing inadequate or excessive concentrations of the amino acid under study were found to be reluctant to feed actively on these diets. These groups of fish, however, were carefully fed to avoid diet rejection by the entire population as a result of the schooling behaviour of carp.

A. Protein Requirement

Experiment 1 :

The aim of this experiment was to determine the dietary protein requirement of fingerling carp at a water temperature of 20°C, prior to investigation of the amino acid requirements. Table 18 summarises growth performance of carp fed five dietary protein concentrations (180.6, 243.2, 281.7, 334.4 and 389.3 g/kg, represented by diets 1P1, 1P2, 1P3, 1P4 and 1P5 respectively). Fish maintained on the highest protein level (diet 1P5) had significantly ($P<0.05$) higher growth rates than those fed on the remaining diets. Fish given diet 1P5 also converted it most efficiently ($P<0.05$), although there was no significant ($P>0.05$) difference in daily DM intake of fish fed the various dietary protein levels.

When plotted against daily protein intake, daily weight gain

TABLE - 18

Experiment 1 - Effect of varying dietary protein levels on the growth, DM intake and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet	Dietary protein level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
1P1	180.6	0.18	0.74	0.25
1P2	243.2	0.20	0.73	0.28
1P3	281.7	0.21	0.74	0.29
1P4	334.4	0.21	0.72	0.29
1P5	389.3	0.28	0.72	0.39
S.E.M.		0.016	0.005	0.016
d.f.		4	4	4

Number of fish per replicate = 15

and daily protein deposition showed a linear relationship when regression lines were fitted to the data (Figs. 10 and 11 respectively). These relationships were described by the following equations:

(i) $Y = 2.6468 + 0.0772X$

where Y is the weight gain (g/fish/d) and X the protein intake (g/fish/d)

(ii) $Y = 0.0967 + 0.0033X$

where Y is the protein deposition (mg/fish/d) and X the protein intake (mg/fish/d).

Maximum weight gain of 0.28 g/fish/d (Fig. 10) and protein deposition of 42.50 mg/fish/d (Fig. 11) occurred within the group consuming 281.2 mg protein/fish/d which was supplied by a dietary protein level of 389.3 g/kg (diet 1P5).

In Table 19 the carcass composition of fish fed the various dietary protein concentrations was compared with that of an initial sample of fish taken at the beginning of the experiment. The fat content of fish offered diet 1P1, containing the lowest dietary protein level, was significantly ($P<0.05$) higher than that of the initial sample, whereas the fat content of fish fed the other diets was not significantly ($P>0.05$) affected when compared with the initial sample. There was a significant ($P<0.05$) reduction in fat content in fish offered diets 1P2 and 1P3 as compared with those given diet 1P1. This reduction in fat content was highly

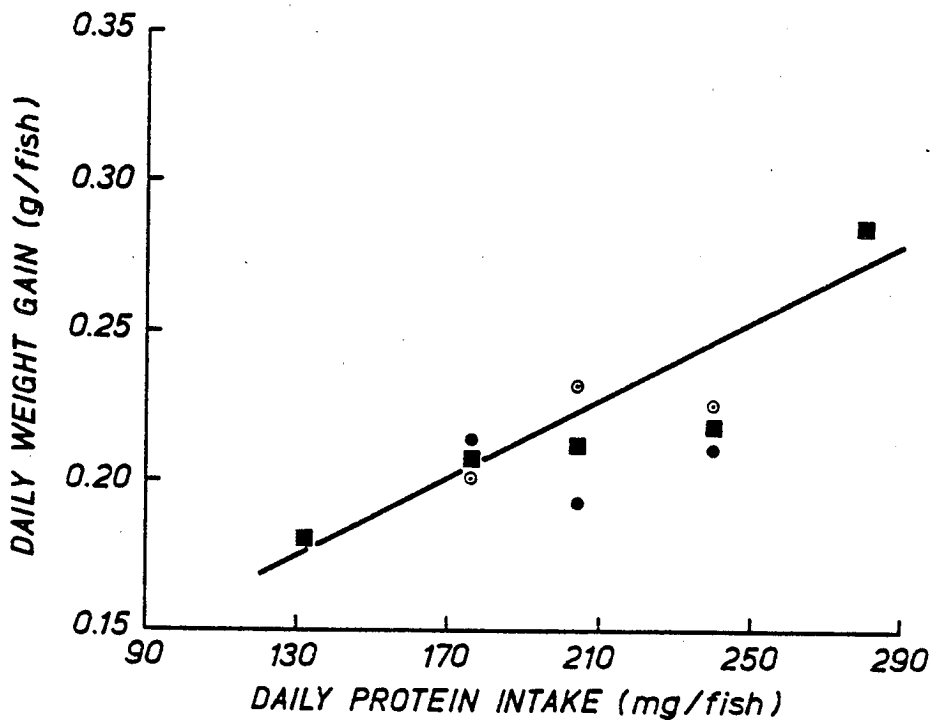


Fig. 10 Daily weight gain (g/fish) and daily protein intake (g/fish) of young carp fed different protein levels and maintained at a water temperature of 20°C. ■, represents the mean of replicate 1 (⊙) and 2 (●)
Number of fish per replicate =15

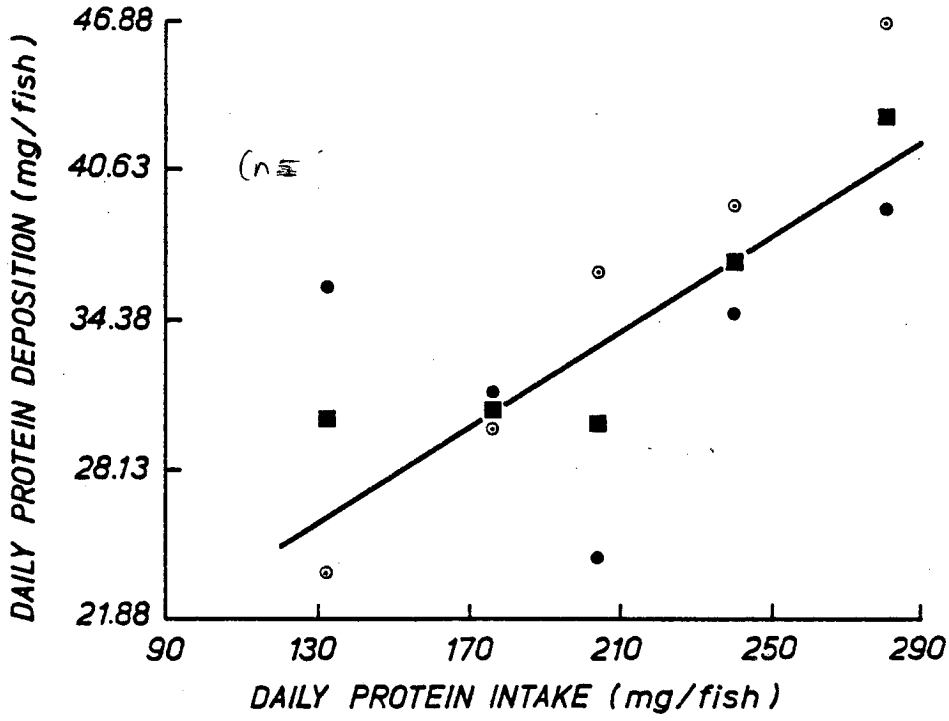


Fig. 11 Exp. 1 Daily protein intake (mg/fish) and daily protein deposition (mg/fish) of young carp fed different protein levels and maintained at a water temperature of 20°C. ■, represents the mean of replicate 1 (⊙) and 2 (●) Number of fish per replicate = 5

TABLE - 19

Experiment 1 - Terminal composition in terms of dry matter, protein, fat, ash and GE of carp fed varying levels of dietary protein at a water temperature of 20°C.

Diet	Dietary protein level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
1P1	180.6	277.8	524.8	277.4	115.2	23.7
1P2	243.2	262.6	554.3	232.6	128.1	23.0
1P3	281.7	259.0	545.6	226.2	131.1	22.8
1P4	334.4	261.4	565.0	218.6	135.2	22.6
1P5	389.3	258.2	557.0	218.2	133.4	23.0
Initial Sample[a]		266.5	528.6	241.2	142.0	22.3
S.E.M.		7.36	12.12	7.95	5.57	0.20
d.f.		6	6	6	6	6

[a] sample of fish taken at the beginning of the experiment

Number of fish per replicate = 5

significant ($P<0.01$) in the groups maintained on diets 1P4 and 1P5. No significant ($P>0.05$) differences in fat content were observed in groups offered diets 1P2, 1P3, 1P4 and 1P5. The DM, protein, ash and GE content of fish fed the various dietary protein levels were not significantly ($P>0.05$) different from those of the initial sample. Nor was there any significant (>0.05) difference within the treatments.

As shown in Table 20, the different concentrations of dietary protein had no significant ($P>0.05$) effect on carcass deposition of fat, ash or GE, or on the efficiency of protein and GE deposition. However, fish fed the lowest level of dietary protein (diet 1P1) had the highest fat deposition and highest efficiency of protein deposition.

Under the present experimental conditions, it was found that maximum growth rate occurred when carp consumed a diet containing 389.3 g protein/kg. A daily intake of 281.2 mg protein/fish resulted in maximum weight gain and maximum protein deposition.

B. Interaction between Dietary Protein and Lysine Concentrations

Experiment 2 :

The aim of this experiment was to investigate, at 20°C, the dietary lysine requirements of fingerling carp at two levels of dietary protein. Table 21 summarises the growth performance of

TABLE - 20

Experiment 1 - Daily carcass deposition of fat, ash and GE in carp fed on diets containing different levels of dietary protein at a water temperature of 20°C.

Diet	Dietary protein level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
1P1	180.6	23.90	1.23	1.70	0.23	0.14
1P2	243.2	10.00	3.54	1.32	0.17	0.11
1P3	281.7	7.90	4.16	1.23	0.15	0.10
1P4	334.4	6.90	5.56	1.25	0.15	0.10
1P5	389.3	9.70	7.05	1.67	0.15	0.13
S.E.M.		3.890	1.280	0.255	0.030	0.020
d.f.		4	4	4	4	4

Number of fish per replicate = 5

TABLE - 21

Experiment 2 - Effect of feeding two dietary protein levels and three lysine concentrations on the growth rate, DM intake and efficiency of food conversion of carp at a water temperature of 20°C.

Diet	Dietary protein level g/kg DM	Dietary lysine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
2P1L0	216.4	11.8	0.23	0.76	0.30
2P1L1	222.1	13.9	0.19	0.77	0.25
2P1L2	215.8	16.1	0.18	0.77	0.23
2P2L0	307.3	12.6	0.23	0.70	0.33
2P2L1	294.8	14.8	0.25	0.68	0.37
2P2L2	297.1	16.6	0.29	0.72	0.40
S.E.M					
Protein			0.009	0.006	0.014
Lysine			0.011	0.071	0.017
Protein x Lysine			0.016	0.010	0.024
d.f.					
Protein			1	1	1
Lysine			2	2	2
Protein x Lysine			2	2	2

Number of fish per replicate = 15

carp fed two dietary protein levels of 218 (2P1) and 389 g/kg (2P2) (each value representing the mean of 3 replicate groups) and three dietary lysine concentrations (12, 14 and 16 g/kg, represented by L0, L1 and L2 respectively) in factorial combination. In general, an increase in the dietary protein level resulted in a significant ($p<0.05$) improvement in daily weight gain, DM intake and efficiency of food utilisation, whereas the dietary lysine concentrations did not significantly ($P>0.05$) improve the growth performance of carp. Nevertheless, a statistical analysis of the growth data revealed a significant ($p<0.05$) interaction between dietary protein levels and lysine concentrations. This relationship was not significant ($P>0.05$) in respect of the DM intake and efficiency of food conversion.

At the lower dietary protein level, a marked growth retardation occurred with increased dietary lysine concentrations. Fish fed on the basal diet (2P1L0), containing 216.4 g protein and 11.8 g lysine/kg, exhibited a maximum daily weight gain of 0.23 g/fish. Conversely, at the higher dietary protein level (389 g/kg DM), dietary lysine additions resulted in a marked improvement in the growth performance of the fish. As shown in Table 21, best growth rate and efficiency of food conversion were observed in the group fed the diet containing 297.1 g protein and 16.6 g lysine/kg DM.

Statistical comparison among groups fed on the three different dietary lysine concentrations at both dietary protein levels showed a significant ($P<0.05$) increase in weight gain in fish receiving the higher protein diets, 2P2L1 and 2P2L2, which contained 14.8 and

16.6 g lysine per kg DM respectively. However, no significant ($P>0.05$) differences in the growth performance were found between fish fed diet 2P2L0 and any of those maintained on the lower protein diets (2P1L0, 2P1L1 and 2P1L2).

The relationship between daily lysine intake and daily weight gain of fish fed the various experimental diets is illustrated in Fig. 12. The growth rate of the fish was positively correlated with increasing lysine intake at the higher protein level and negatively correlated at the lower protein level. Similar trends were observed when daily protein deposition was plotted against daily lysine intake (Fig. 13). However, maximum daily weight gain of 0.29 g/fish and daily protein deposition of 54.38 mg/fish was observed within the group maintained on diet 2P2L2, the individuals of which consumed an average of 11.7 mg lysine/fish/day.

The terminal composition of fish fed the various experimental diets showed no significant ($P>0.05$) difference in DM, protein, fat, ash and GE contents as compared with that of the initial sample (Table 22) and among the various fish groups.

Table 23 summarises the data on carcass deposition of fat, ash and GE, and the efficiency of protein and GE deposition. The dietary protein, lysine, and the combined protein and lysine levels had no significant ($P>0.05$) effect on the deposition of fat, ash, and efficiency of protein and GE deposition, whereas GE deposition showed a significant ($P<0.05$) improvement when the dietary protein level was increased. The fish fed on the higher protein diets

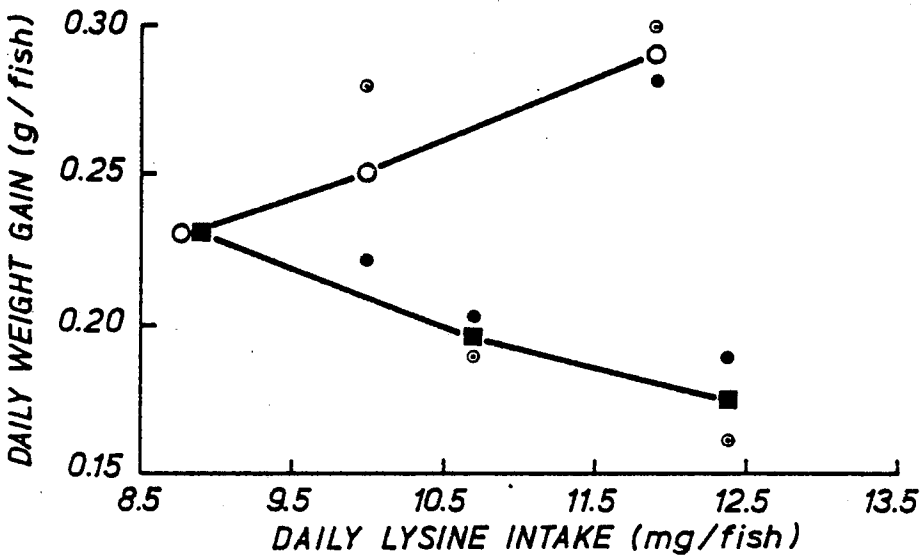


Fig. 12 Relationship between daily weight gain (g/fish) and Exp. 2 daily lysine intake (mg/fish) of young carp fed three concentrations of lysine and two levels of protein (218, 300 g/kg diet) and maintained at a water temperature of 20°C. ■, O, represents the mean of replicate 1 (⊙) and 2 (●) for the experimental diets containing protein levels of 218 (■) and 300 (O) g/kg DM.
Number of fish per replicate= 15

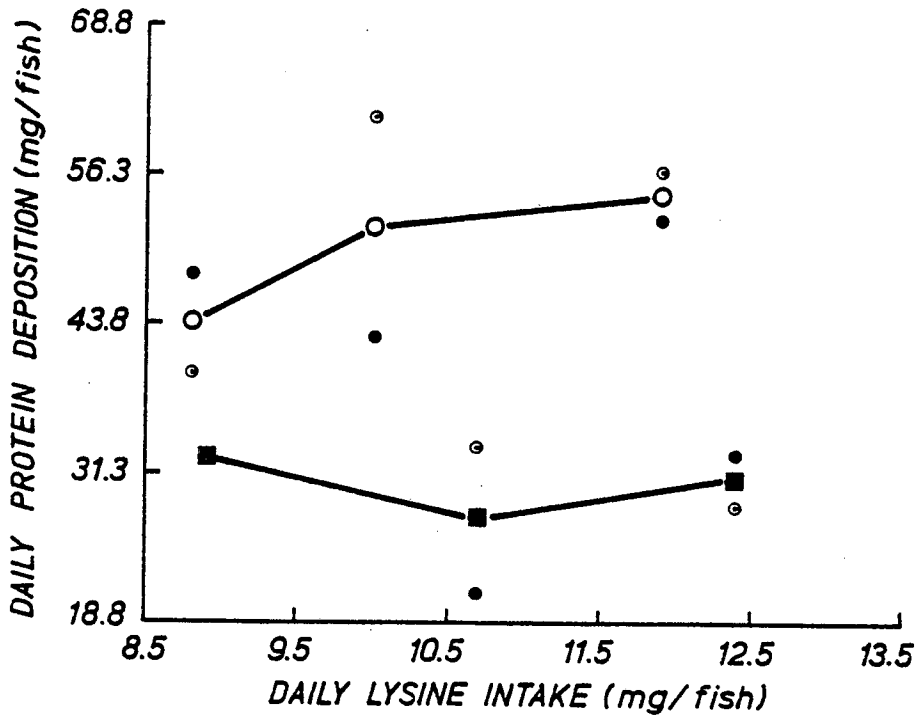


Fig. 13 Exp. 2 Daily lysine intake (mg/fish) and daily protein deposition (mg/fish) of young carp fed three concentrations of lysine and two levels (218, 300 g/kg) of dietary protein, and maintained at a water temperature of 20°C. ■, O, represents the mean of replicate 1 (⊙) and 2 (●) for the experimental diets containing protein levels of 218 (■) and 300 (O) g/kg DM.
Number of fish per replicate = 15

TABLE - 22

Experiment 2 - Terminal carcass composition in terms of dry matter, protein, fat, ash and gross energy of carp fed on diets containing 2 levels of protein and 3 concentrations of dietary lysine in factorial combination at a water temperature of 20°C.

Diet	Dietary protein level g/Kg DM	Dietary lysine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
2P1L0	216.4	11.8	254.8	520.9	227.6	134.3	22.8
2P1L1	222.1	13.9	246.3	541.8	221.0	149.0	22.1
2P1L2	215.8	16.1	253.7	546.1	229.3	139.0	22.9
2P2L0	307.3	12.6	261.2	551.9	229.1	125.5	23.3
2P2L1	294.8	14.8	266.0	559.1	242.2	119.2	23.4
2P2L2	297.1	16.6	283.2	516.1	238.8	119.8	23.9
Initial Sample (I.S.)			237.9	549.2	225.5	153.7	22.6
S.E.M.							
I.S. x Protein			6.06	10.16	1.48	4.00	0.26
I.S. x Lysine			6.42	10.79	1.57	4.24	0.28
I.S. x Protein x Lysine			7.42	12.45	1.80	4.89	0.32
d.f.							
I.S. x Protein			1	1	1	1	1
I.S. x Lysine			2	2	2	2	2
I.S. x Protein x Lysine			2	2	2	2	2

Number of fish per replicate = 15

TABLE - 23

Experiment 2 - Carcass deposition of fat, ash and GE in carp fed on diets containing five levels of protein and three concentrations of dietary lysine at a water temperature of 20°C.

Diet	Dietary protein level g/kg DM	Dietary lysine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
2P1L0	216.4	11.8	17.80	6.12	1.68	0.20	0.13
2P1L1	222.1	13.9	5.10	7.00	1.09	0.16	0.08
2P1L2	215.8	16.1	10.60	5.28	1.39	0.19	0.55
2P2L0	307.3	12.6	18.90	4.72	1.96	0.22	0.16
2P2L1	294.8	14.8	22.50	4.33	2.24	0.26	0.20
2P2L2	297.1	16.6	38.50	7.80	3.03	0.26	0.24
S.E.M.							
	Protein		2.914	0.574	0.154	0.018	0.118
	Lysine		3.571	0.702	0.187	0.021	0.137
	Protein x Lysine		5.057	0.993	0.265	0.030	0.137
d.f.							
	Protein		1	1	1	1	1
	Lysine		2	2	2	2	2
	Protein x Lysine		2	2	2	2	2

Number of fish per replicate = 15

exhibited increases in fat and GE deposition as the dietary lysine concentration increased, though this increase was not significant ($P>0.05$). Maximum fat, ash and GE deposition and highest efficiency of protein and GE deposition was exhibited by the group receiving diet 2P2L2.

The results showed that, at the lower dietary protein level, increasing the lysine concentrations had an inhibitory effect on the growth performance of carp within the range tested. In contrast, increasing the lysine concentrations at the higher dietary protein level improved growth performance. The requirement of carp for dietary lysine is therefore lower (12.1 g/kg) at the lower dietary protein level of 218 g/kg, and tends to be higher (20.6 g/kg) at the higher dietary protein concentration (298/kg). When these requirements are expressed in terms of dietary protein, the lysine requirement of carp is 54.4 and 69.2 g/kg respectively for the lower and higher dietary protein levels tested. At the lower and higher dietary protein concentrations, intake levels of 8.7 and 11.7 mg lysine/fish/d respectively resulted in maximum growth rate and protein deposition. Excessive levels of lysine intake at the lower dietary protein concentration resulted in growth retardation.

C. Lysine Requirements

Three experiments were conducted to estimate the requirement of fingerling carp for dietary lysine. Experiments 3 and 4 were planned to investigate the requirements of carp at 20°C, using

recycled and non-recycled water systems respectively, whereas the objective of experiment 5 was to assess the requirement at 25°C using a non-recycled water system.

1. Experiment 3 :

The response of carp (Table 24), reared in a recycled water system at 20°C, to five dietary lysine concentrations of 12.1, 14.2, 16.3, 18.5, 20.6 and 22.7 g/kg, was tested at an average protein level of 450 g/kg DM. These diets were presented as 3L0, 3L1, 3L2, 3L3, 3L4 and 3L5 respectively. The addition of 2 g lysine/kg (diet 3L1) to the basal diet (3L0) increased the growth rate, DM intake and efficiency of food conversion. However, further dietary lysine additions failed to enhance performance, which indicates that the lysine concentration in the basal diet was sub-optimal for carp.

Growth response curves were obtained when daily weight gain (Fig. 14) and daily protein deposition (Fig. 15) were plotted against daily lysine intake of fish groups offered the various experimental diets. As can be seen, a maximum daily weight gain of 0.26 g/fish and daily protein deposition of 34.06 mg/fish occurred when each fish consumed 6.0 mg lysine per day.

Table 25 sets out the results of the terminal composition of fish fed the different dietary lysine concentrations and those of a sample of fish taken at the beginning of the experiment. No significant ($P>0.05$) differences were found either in the contents

TABLE - 24

Experiment 3 - Effect of varying dietary lysine levels on the growth rate, DM intake and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet[a]	Dietary lysine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
3L0	12.1	0.16	0.35	0.47
3L1	14.2	0.26	0.42	0.62
3L2	16.3	0.25	0.43	0.60
3L3	18.5	0.26	0.43	0.61
3L4	20.6	0.26	0.42	0.64
3L5	22.7	0.27	0.43	0.63
S.E.M.		0.023	0.011	0.020
d.f.		5	5	5

[a] protein level is 452 g/kg DM

Number of fish per replicate = 17

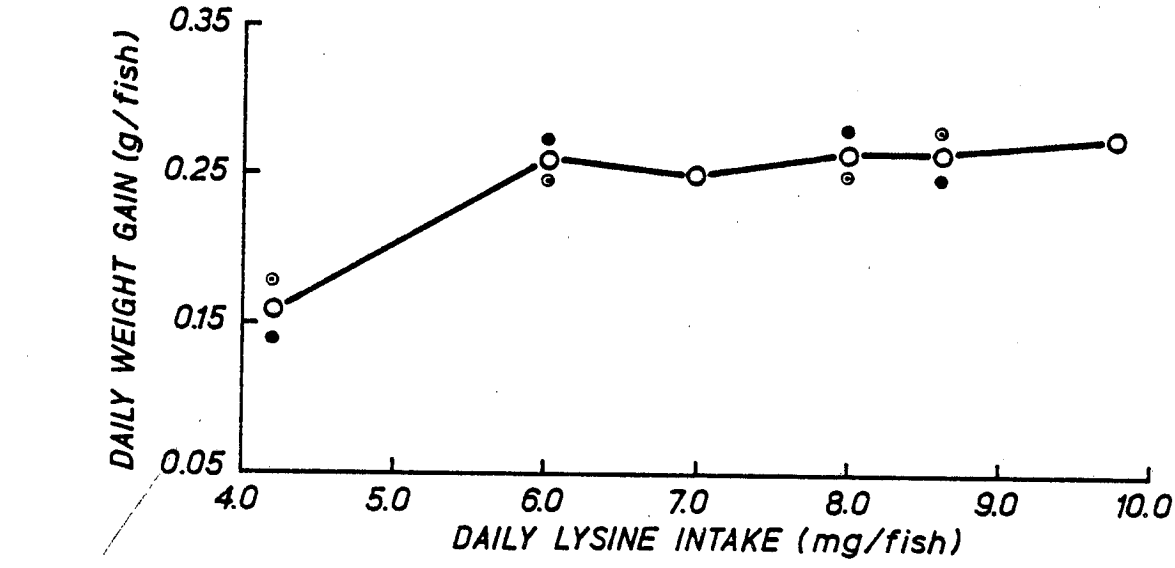


Fig. 14 Exp. 3 Daily weight gain (g/fish) and daily lysine intake(mg /fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●) Number of fish per replicate = 17.

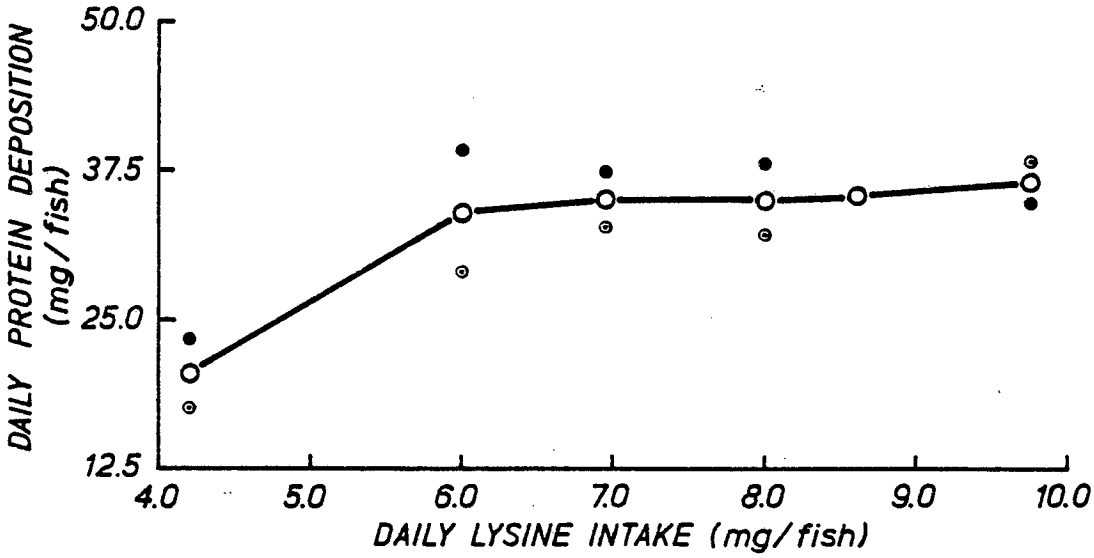


Fig. 15 Exp. 3 Daily lysine intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 7

TABLE - 25

Experiment 3 - Terminal composition in terms of dry matter, protein, fat, ash and GE of carp fed on diets containing different levels of dietary lysine at a water temperature of 20°C.

Diet	Dietary lysine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/DM DM
3L0	12.1	252.6	508.1	234.0	128.4	23.2
3L1	14.2	242.4	534.1	217.6	132.5	22.8
3L2	16.3	257.0	516.7	254.6	117.7	24.1
3L3	18.5	245.3	529.9	233.2	122.9	23.6
3L4	20.6	246.7	530.0	239.4	126.9	23.5
3L5	22.7	244.2	535.1	229.4	128.9	23.3
Initial	Sample	241.5	533.1	215.8	143.3	22.9
S.E.M		5.15	13.67	12.50	3.56	0.33
d.f.		7	7	7	7	7

Number of fish per replicate = 7

of DM, protein, fat, ash and GE of the experimental fish groups and those of the initial sample, or within the treatments.

The data presented in Table 26 show the effect of different dietary lysine concentrations on carcass deposition of fat, ash and GE, and on the efficiency of deposition of protein and GE. Fish given diets 3L2, 3L3 and 3L4 showed a significant ($P<0.05$) increase in fat deposition as compared with those offered the basal diet. No significant ($P>0.05$) differences in fat deposition were observed among the groups maintained on diets 3L1 and 3L5, and those on the basal diet. However, maximum fat deposition of 18.28 mg/fish/d was found in the group receiving the diet 3L4. Ash deposition in fish fed on diets 3L1, 3L2, 3L4 and 3L5 was significantly ($P<0.05$) higher than in those on the basal diet. The GE deposition and the efficiency of protein and GE deposition, however, was not significantly ($P>0.05$) improved by the various dietary lysine concentrations.

These results indicate that the lysine requirement of carp, maintained at a water temperature of 20°C, is 14.2 g/kg DM or 31.9 g/kg dietary protein. When the requirement of carp is expressed in terms of daily intake, a level of 6 mg lysine/fish was found to be adequate for optimum growth rate and protein utilisation.

Experiment 4 :

The aim of this experiment was to confirm the results obtained in Experiment 3, using a non-recycled water system. Table 27

TABLE - 26

Experiment 3 - Carcass deposition of fat, ash and GE in carp fed varying levels of dietary lysine at a water temperature of 20°C.

Diet	Dietary lysine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
3L0	12.1	12.5	4.30	1.10	0.13	0.17
3L1	14.2	14.0	7.36	1.43	0.18	0.18
3L2	16.3	22.2	5.84	1.48	0.19	0.18
3L3	18.5	17.3	6.13	1.64	0.18	0.20
3L4	20.6	18.3	7.04	1.63	0.18	0.21
3L5	22.7	16.8	7.32	1.60	0.18	0.20
S.E.M.		1.608	0.452	0.221	0.013	0.026
d.f.		5	5	5	5	5

Number of fish per replicate = 7

TABLE - 27

Experiment 4 - Effect of varying dietary lysine levels on the growth rate, DM intake and efficiency of food conversion of carp at a water temperature of 20°C.

Diet	Dietary lysine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g/gain/g DM intake
4L0	12.5	0.38	0.72	0.53
4L1	14.6	0.53	0.77	0.69
4L2	16.7	0.52	0.75	0.70
4L3	18.8	0.52	0.78	0.68
S.E.M.		0.038	0.010	0.039
d.f.		3	3	3

Number of fish per replicate = 13

summarises the growth performance of carp fed four dietary lysine levels (12.5, 14.6, 16.7 and 18.8 g/kg) represented by diets 4L0, 4L1, 4L2 and 4L3 respectively. A significant ($P<0.05$) increase in weight gain occurred when the basal diet (4L0) was supplemented with 2 g lysine/kg (diet 4L1). Higher additions of dietary lysine did not result in any further weight gain. The daily DM intake and efficiency of food utilisation were also improved by increasing the dietary lysine level from 12.5 to 14.6 g/kg DM, but this difference was not significant ($P>0.05$). These findings indicate that the lysine concentration of the basal diet was inadequate for carp.

Fig. 16 illustrates the growth response of carp in relation to daily lysine intake. A level of 11.0 mg/fish/d was found to be adequate for supporting a maximum growth of 0.53 g/fish/d. However, maximum daily protein deposition of 81.3 mg/fish occurred within the group consuming 12.7 mg/fish/d (see Fig. 17).

All the attributes of carcass composition (Table 28) and carcass deposition of fat and GE, and the efficiencies of protein and GE deposition (Table 29) were not significantly ($P>0.05$) affected by increasing the dietary lysine concentration. A significant ($P<0.05$) reduction in ash deposition (Table 29) was observed in the group fed on the basal diet as compared with those on diets containing higher levels of lysine.

These results confirm the earlier observations of Experiment 3, which indicate that the lysine requirement of carp, at a water temperature of 20°C, is 14.5 g/kg diet or 32.6 g/kg dietary

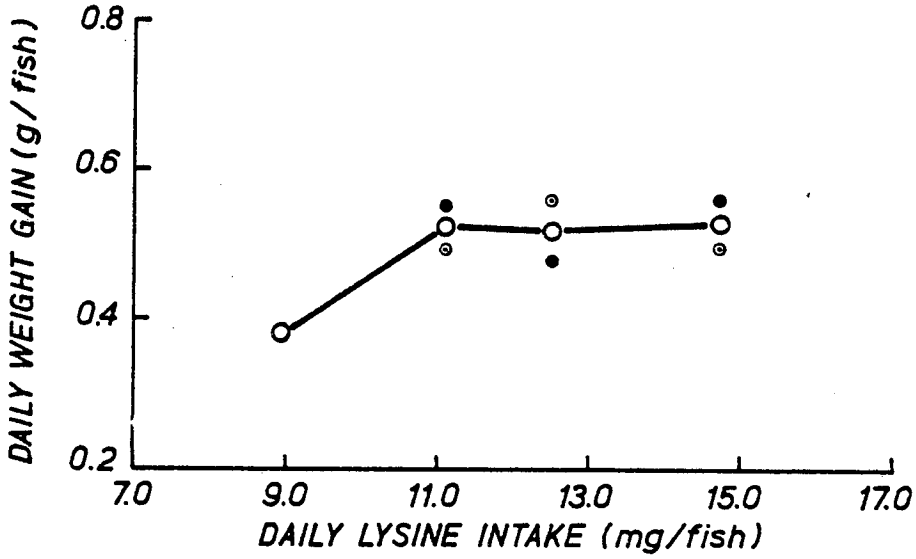


Fig. 16 Daily weight gain (g/fish) and daily lysine intake (mg/fish) Exp. 4 of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●). Number of fish per replicate = 13

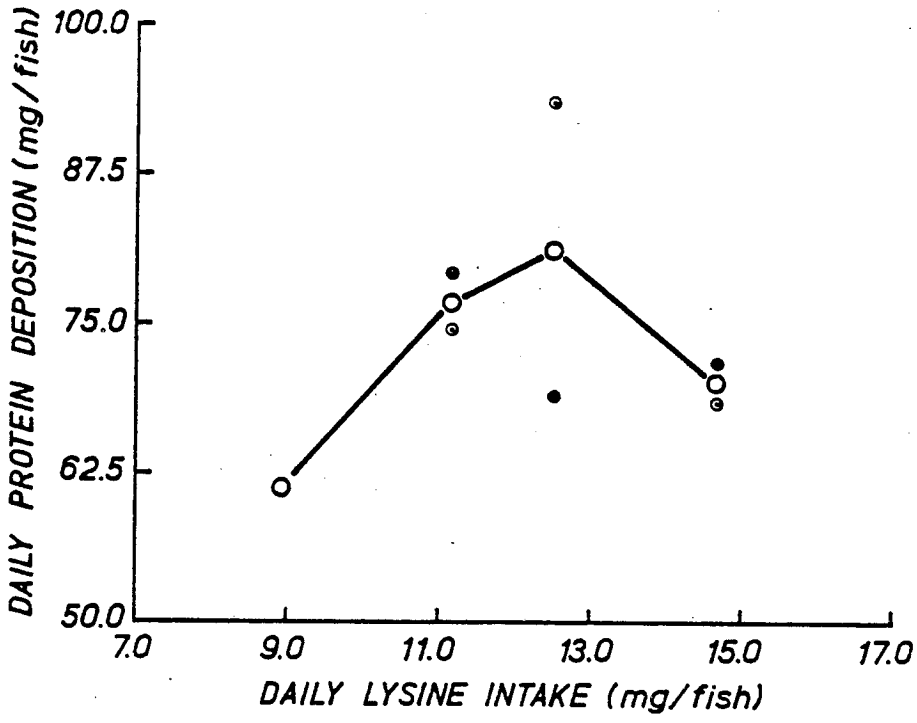


Fig. 17 Exp. 4 Daily lysine intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●). Number of fish per replicate = 13

TABLE - 28

Experiment 4 - Terminal composition of carp fed four dietary lysine levels at a water temperature of 20°C.

Diet	Dietary lysine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
4L0	12.5	249.2	544.6	225.0	111.4	23.6
4L1	14.6	253.4	522.7	235.6	109.2	24.1
4L2	16.7	259.3	522.9	248.4	109.9	24.0
4L3	18.8	252.6	509.8	255.4	112.4	24.0
Initial	Sample	230.7	536.8	228.0	123.4	23.1
S.E.M.		4.87	13.37	10.36	0.19	5.25
d.f.		5	5	5	5	5

Number of fish per replicate = 13

TABLE - 29

Experiment 4 - Carcass deposition of fat, ash and GE in carp fed varying levels of dietary lysine at a water temperature of 20°C.

Diet	Dietary lysine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited / mg intake	Efficiency of GE deposition kJ deposited /kJ intake
4L0	12.5	24.40	10.20	2.70	0.20	0.20
4L1	14.6	37.10	14.04	3.83	0.23	0.27
4L2	16.7	43.50	15.00	4.00	0.24	0.29
4L3	18.8	43.00	14.99	3.72	0.21	0.26
S.E.M.		7.546	0.697	0.419	0.023	0.029
d.f.		3	3	3	3	3

Number of fish per replicate = 13

protein. The daily intake of lysine required to promote optimum growth and protein deposition was found to be 11 mg per fish.

Experiment 5 :

The growth performance of carp maintained in a non-recycled water system at 25°C and fed on six dietary lysine concentrations (12.4, 14.6, 16.7, 18.9, 21.0 and 23.2 g/kg, represented by diets 5L0, 5L1, 5L2, 5L3, 5L4, and 5L5 respectively) is shown in Table 30. Significant ($P<0.05$) responses in weight gain and efficiency of food utilisation occurred on supplementation of the basal diet (5L0) with dietary lysine. These observations indicate that the lysine level in the basal diet was limiting.

The analysis of variance and t-test values showed that there was no significant ($P>0.05$) difference in weight gain between the fish offered diet 5L0 and those given diet 5L1. A significant (<0.05) increase in weight gain occurred within the group fed on diet 5L2, when compared with those fish maintained on diet 5L0. Fish given a higher concentration (diet 5L3, 5L4 and 5L5) of lysine showed a highly significant ($P<0.01$) increase in growth rate as compared with those offered the basal diet. When compared with the group offered diet 5L2, fish given diet 5L5 showed a significant ($P<0.05$) increase in weight gain. However, no significant ($P>0.05$) differences in weight gain were found between the groups maintained on diets 5L3 and 5L4 when the same groups were compared with those given diets 5L2 or 5L5.

TABLE - 30

Experiment 5 - Effect of varying dietary lysine levels on weight gain, DM, intake and efficiency of food conversion of carp maintained at a water temperature of 25°.

Diet	Dietary lysine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
5L0	12.4	0.44	0.88	0.53
5L1	14.6	0.48	0.85	0.64
5L2	16.7	0.54	0.87	0.65
5L3	18.9	0.57	0.88	0.67
5L4	21.0	0.56	0.86	0.66
5L5	23.2	0.62	0.91	0.52
S.E.M.		0.027	0.017	0.042
d.f.		5	5	5

Number of fish per replicate =10

Table 30 shows the data on dry matter intake and efficiency of food conversion within the various fish groups. Analysis of variance revealed no significant ($P>0.05$) effect of the different concentrations of lysine on the DM intake or efficiency of food conversion. However, optimum efficiency of food utilisation occurred within the group that received diet 5L2.

The effects of daily lysine intake on the daily weight gain and daily protein deposition of carp are shown in Fig. 18 and Fig. 19 respectively. Maximum daily weight gain of 0.57 g/fish was found with the group consuming 16.7 mg lysine/fish/d. An intake level of 18.0 mg lysine/fish/d was found to be adequate for a maximum daily protein deposition of 95.4 mg/fish.

Dry matter, fat, ash and GE contents of the fish fed on the various experimental diets, in comparison with those of the initial sample, are shown in Table 31. The DM content of fish given the various dietary lysine levels, except those fed diet 5L3, was substantially ($P<0.01$) lower than that of the initial sample. The fish given diet 5L3 showed a significant ($P<0.05$) increase in DM content as compared with those offered diets 5L0 and 5L1. No significant ($P>0.05$) differences were found in protein, fat, and GE content of fish fed on the various dietary lysine concentrations and those of the initial sample. The ash content of fish given the various dietary lysine levels, except those offered diet 5L4, was significantly ($P<0.05$) lower than that of the initial sample. A statistical comparison among the various experimental fish groups revealed no significant ($P>0.05$) differences in their ash content.

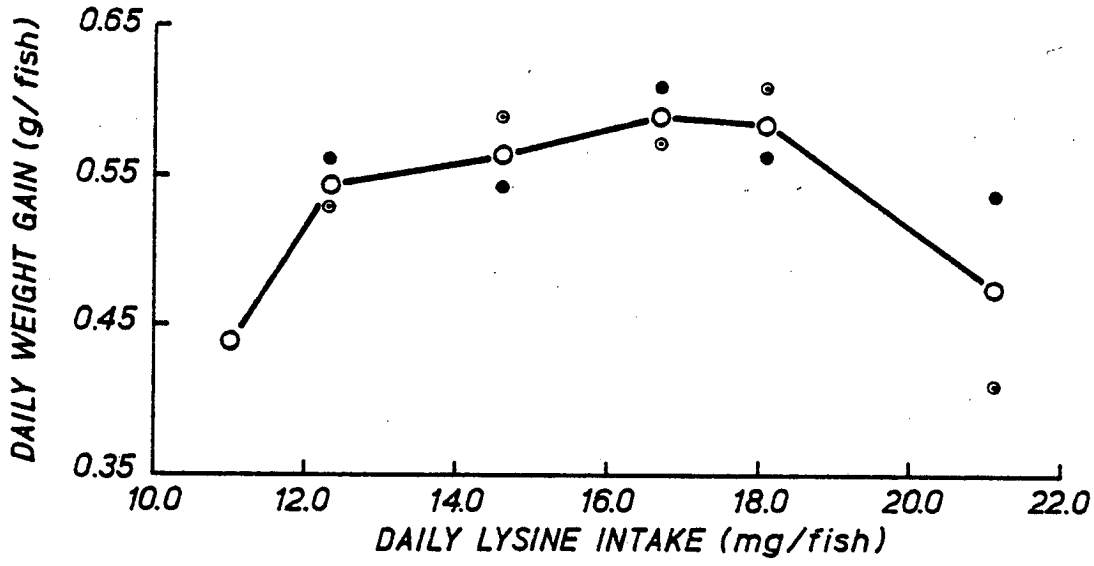


Fig. 18 Daily lysine intake (mg/fish) and daily weight gain (g/fish) of young carp maintained at a water temperature of 25°C. O, represents the mean of replicate 1 (⊙) and 2 (●). Number of fish per replicate = 10.

replicates

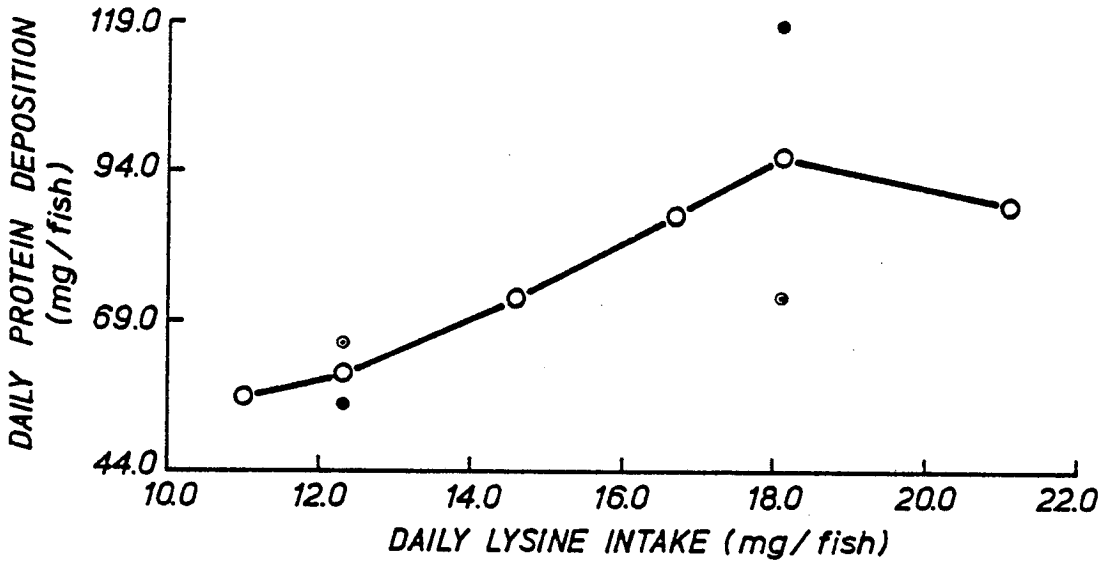


Fig. 19 Exp. 5 Daily lysine intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 25°C. **O**, represents the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 10

TABLE - 31

Experiment 5 - Terminal composition of carp fed on five dietary lysine levels at a water temperature of 25°C.

Diet	Dietary lysine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
5L0	12.4	238.8	558.6	241.0	108.9	23.0
5L1	14.6	242.9	540.1	259.0	113.5	24.0
5L2	16.7	248.1	547.1	256.8	104.2	23.8
5L3	18.9	256.6	561.9	258.6	100.5	24.3
5L4	21.0	246.4	532.3	273.4	108.7	24.2
5L5	23.2	247.8	561.3	231.6	108.8	23.8
Initial Sample		269.4	508.2	299.4	116.7	24.3
S.E.M.		2.89	8.68	8.98	2.19	0.24
d.f.		7	7	7	7	7

Number of fish per replicate = 10

Table 32 summarises the results of carcass deposition of fat, ash and GE, and the efficiency of protein and GE deposition of carp. Fat deposition and the efficiency of protein deposition were not significantly ($P>0.05$) affected by increasing the dietary lysine concentrations. However, there was a gradual increase in fat deposition and the efficiency of protein deposition with increased lysine concentrations of up to 21.03 g/kg diet, after which a fall in efficiency of protein deposition was observed. Ash and GE deposition, and the efficiency of GE deposition increased significantly ($P<0.05$) with dietary lysine additions. The deposition of ash was significantly ($P<0.05$) higher in fish given diets 5L3 and 5L4 as compared with those offered the basal diet. Fish maintained on diet 5L5 showed a highly ($P<0.01$) significant increase in carcass deposition of ash as compared with those fed on diet 5L0. However, this increase in ash deposition was just significant ($P<0.05$) when compared with the groups given diets 5L1, 5L2 and 5L3. There were no significant ($P>0.05$) differences in ash deposition of fish given diets 5L4 and 5L5.

A significant ($P<0.05$) increase in carcass deposition of GE was observed within the group given diet 5L2, as compared with those offered the basal diet. This increase in GE deposition, however, was highly significant ($P<0.01$) in the groups given diets 5L3, 5L4 and 5L5, and still significant ($P<0.05$) even when compared with those maintained on diet 5L1. However, no significant ($P>0.05$) differences in GE deposition among groups fed on diets 5L2, 5L3, 5L4 and 5L5 were observed. The analysis of variance and t-test of the efficiency of GE deposition data showed similar results as

TABLE - 32

Experiment 5 - Carcass deposition of protein, ash and GE of carp fed on diet containing graded concentrations of dietary lysine at a water temperature of 25°C.

Diet	Dietary lysine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
5L0	12.4	13.60	8.70	1.88	0.16	0.11
5L1	14.6	21.10	11.24	2.42	0.18	0.15
5L2	16.7	25.20	10.91	2.84	0.21	0.17
5L3	18.9	30.10	11.64	3.37	0.24	0.20
5L4	21.0	30.70	12.55	3.03	0.27	0.19
5L5	23.2	23.80	14.47	3.33	0.24	0.19
S.E.M.		3.402	0.704	0.152	0.029	0.011
d.f.		5	5	5	5	5

Number of fish per replicate = 10

found with GE deposition, except that the increase in the efficiency of GE deposition was close to significance ($P<0.05$) in fish given diet 5L4, as compared with those maintained on diet 5L1.

These results suggest that a dietary lysine level of 18.9 g/kg or 46.7 g/kg dietary protein is adequate for maximum growth rate and protein deposition of carp maintained at a water temperature of 25°C. However, a dietary lysine level of 16.7 g/kg or 41.3 g/kg dietary protein is considered best for optimum food utilisation and protein deposition. The daily intake of lysine required to promote maximum growth and to ensure optimum utilisation of protein was found to be 16.7 mg per fish. Excessive intake of lysine, under the present experimental condition, caused a growth depression and lowered the carcass deposition of protein.

D. Sulphur Amino Acid Requirements

The aims of the following experiments were to determine the requirement of carp for dietary methionine using the DL- and L-isomer of methionine and to study the sparing action of cystine on methionine requirements.

Experiment 6 :

The effects of varying concentrations of dietary methionine on the growth performance of carp are shown in Table 33. The addition of methionine (DL-form) to the basal diet (6M0) resulted in a progressive increase in weight gain. Fish offered diets 6M1 and

TABLE - 33

Experiment 6 - Effect of varying dietary methionine concentrations on the growth rate, DM intake and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet[a,b]	Dietary methionine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
6M0	6.1	0.14	0.40	0.34
6M1	7.7	0.19	0.41	0.51
6M2	9.4	0.22	0.43	0.52
6M3	11.0	0.29	0.50	0.58
6M4	12.6	0.28	0.51	0.55
S.E.M.		0.018	0.012	0.554
d.f.		4	4	4

[a] [b] methionine supplements added as the DL-racemic mixture containing 2-4 g cystine/kg DM

Number of fish per replicate = 15

6M2, containing 7.7 and 9.4 g methionine/kg DM respectively, grew significantly ($P<0.05$) heavier than those on the basal diet (6M0) containing 6.1 methionine /kg DM. On the other hand, fish given diets 6M3 and 6M4, which contained 11.0 and 12.6 g methionine/kg DM respectively, showed a significant increase in weight gain as compared with the group offered the basal diet ($P<0.01$), or those fed on diets 6M1 and 6M2. The DM intake of fish fed diets 6M1 and 6M2 was also significantly ($P<0.05$) higher than those receiving the basal diet. Fish fed on the diets 6M3 and 6M4 consumed significantly ($P<0.05$) more DM than those on the basal diet. The efficiency of food utilisation improved gradually with increased dietary methionine concentrations, though this improvement was not statistically significant ($P>0.05$). Maximum efficiency of food conversion (0.58) occurred in the group given diet 6M3.

When the daily weight gain of fish fed the various dietary methionine concentrations was plotted against daily intake of sulphur amino acids (methionine and cystine), gradual increases in weight gain of up to 0.29 g/fish/d occurred as the daily intake of sulphur amino acids increased to 6.7 mg/fish (Fig. 20). At this level, which was provided by diet 6M3, the growth curve reached its plateau and further increases in sulphur amino acids intake did not improve daily weight gain. A similar trend was observed when daily carcass protein deposition was plotted against daily intake of sulphur amino acids (Fig. 21). It was found that an intake of 6.7 mg sulphur amino acids/fish/d was adequate for maximum growth and optimum utilisation of protein.

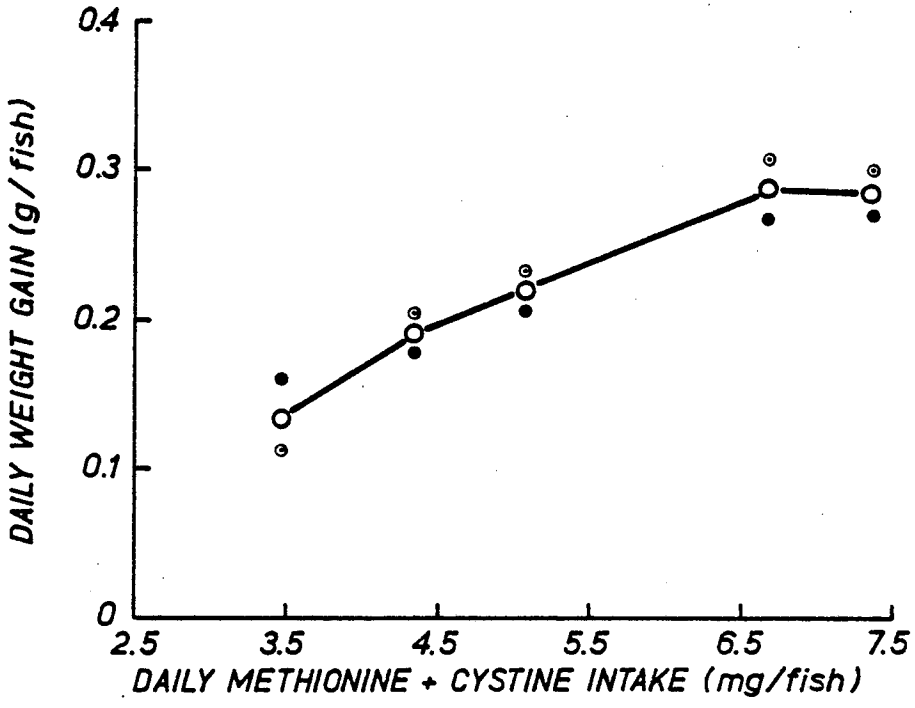


Fig. 20 Exp. 6 Daily weight gain (g/fish) and daily methionine + cystine intake (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●). The DL-isomer of methionine was used as a supplement.
Number of fish per replicate = 15

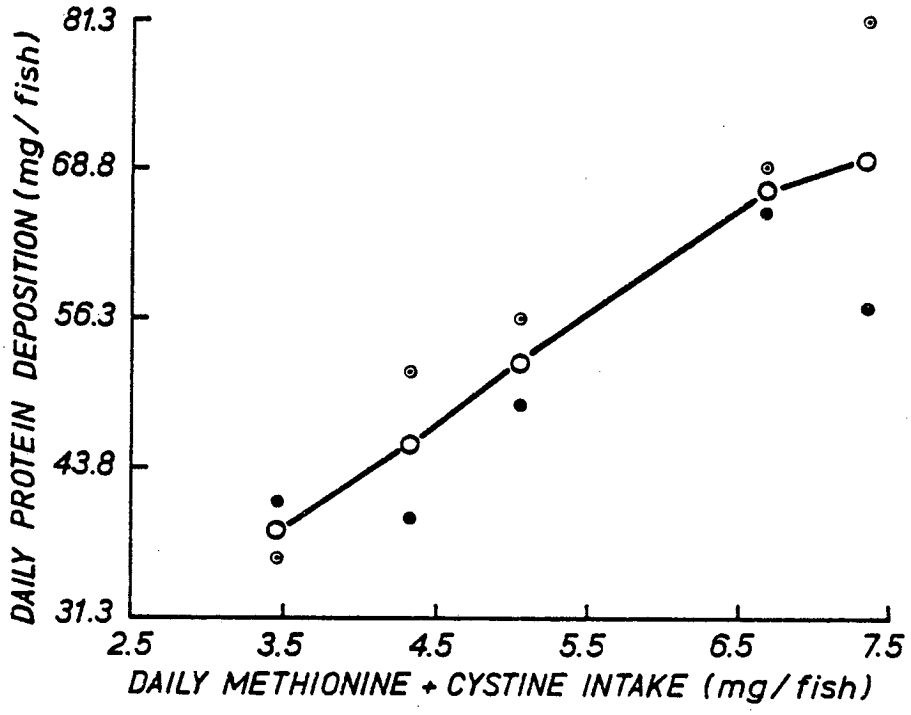


Fig. 21 Exp. 6 Daily methionine + cystine intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 5

Table 34 compares the carcass composition of fish fed on the various dietary methionine concentrations with that of the initial sample. The statistical analysis showed a significant ($P<0.05$) increase in ash content of the fish fed on diet 6M1 as compared with that of the other experimental groups. No significant ($P>0.05$) differences were found in DM, protein, fat and GE content of the experimental fish groups, even when compared with those of the initial sample.

The data given in Table 35 indicates that the different concentrations of dietary methionine induced no significant ($P>0.05$) effects on carcass deposition of fat, ash and GE, or on the efficiency of protein and GE deposition. The optimum efficiency of protein and GE deposition occurred wthin the group given diet 6M3 containing 11.0 g methionine/kg DM.

The results obtained in this experiment indicate that the dietary methionine requirement of carp, reared at a water temperature of 20°C, is 11.0 g/kg in the presence of 2.4 g cystine/kg DM, or 28.9 g/kg dietary protein in the presence of 6.3 g cystine/kg dietary protein. A daily intake of 6.7 mg sulphur amino acids/fish was considered to be adequate for maximum growth and optimum utilisation of protein.

Experiment 7 :

Table 36 compares the growth performance of carp offered diets supplemented with various (four) levels of L-methionine with those

TABLE - 34

Experiment 6 - Terminal composition in terms of dry matter, protein, fat, ash and GE of carp fed varying concentrations of dietary methionine at a water temperature of 20°C.

Diet	Dietary methionine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
6M0	6.1	252.7	593.3	208.0	130.6	23.3
6M1	7.7	249.3	596.1	188.0	188.4	21.9
6M2	9.4	248.0	607.6	172.0	129.6	22.4
6M3	11.0	262.2	582.1	219.0	131.1	22.7
6M4	12.6	260.2	601.0	208.6	132.7	23.2
Initial	Sample	237.9	547.7	210.8	153.7	22.6
S.E.M.		3.25	14.82	13.69	9.31	0.52
d.f.		6	6	6	6	6

Number of fish per replicate = 5

TABLE - 35

Experiment 6 - Carcass deposition of fat, ash and GE in carp fed on diets containing graded levels of methionine at a water temperature of 20°C.

Diet	Dietary methionine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
6M0	6.1	9.51	1.19	1.29	0.25	0.19
6M1	7.7	5.89	18.86	1.13	0.26	0.14
6M2	9.4	2.87	2.86	1.42	0.30	0.18
6M3	11.0	23.43	7.72	2.26	0.34	0.25
6M4	12.6	19.37	7.99	2.36	0.35	0.26
S.E.M.		5.467	3.557	0.259	0.029	0.036
d.f.		4	4	4	4	4

Number of fish per replicate = 5

TABLE - 36

Experiment 7 - Effect of varying dietary methionine levels on the growth rate, DM intake and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet[a,b]	Dietary methionine level g/kg DM	Daily weight gain g/fish	DM intake g/fish	Efficiency of food conversion g gain/g DM intake
7M0	6.1	0.08	0.20	0.49
7M1	7.6	0.11	0.32	0.33
7M2	9.3	0.16	0.36	0.45
7M3	10.9	0.18	0.39	0.45
7M4	12.5	0.14	0.34	0.41
S.E.M.		0.015	0.047	0.087
d.f.		4	4	4

[a] [b] methionine supplements added as the L-isomer containing 2.4 g cystine/kg DM

Number of fish per replicate = 13

fed on the basal diet (7M0) containing 6.1 g methionine/kg DM. The fish given diets 7M2, 7M3 and 7M4, containing 9.3, 10.9 and 12.5 g methionine/kg respectively, grew significantly ($P<0.05$) heavier than those offered the basal diet. However, the growth rate of fish fed on diet 7M3 was significantly ($P<0.05$) faster than those on diet 7M1 which contained 7.5 g methionine/kg DM. There were no appreciable differences in weight gain among the groups receiving diets 7M1 and 7M2, and 7M2 and 7M3. The fish fed on the highest dietary methionine concentration (diet 7M4) exhibited a depressed growth rate, although this reduction in weight gain was not significantly different from those fed on other supplemented diets. The DM intake and efficiency of food conversion of the various fish groups was not significantly ($P>0.05$) increased by increasing the dietary methionine concentrations. Maximum efficiency of food conversion occurred within the group given diet 7M2, but the fish fed on diet 7M4, containing the highest dietary methionine levels, showed a marked reduction in growth rate and efficiency of food utilisation.

When daily weight gain (Fig.22) and daily protein deposition (Fig. 23) were plotted against sulphur amino acids intake, growth response curves were obtained. An increase in daily sulphur amino acid intake up to 4.2 mg/fish caused an increase in daily weight gain from 0.08 to 0.16 g/fish, and daily protein deposition from 6.81 to 20.75 mg/fish. At an intake level of 5.1 mg sulphur amino acids/fish/d, there was an abrupt reduction in weight gain and protein deposition which was followed by an increase at intake level of 5.3 mg sulphur amino acids/fish/d. However, maximum

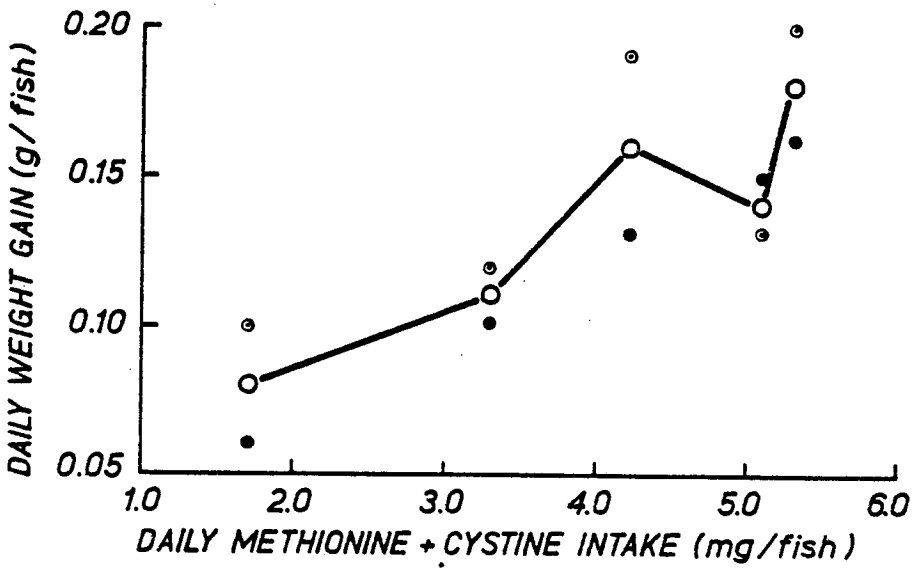


Fig. 22 Exp. 7 Daily weight gain (g/fish) and daily methionine + cystine intake (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●). Methionine supplements were added as L-isomer. Number of fish per replicate = 3.

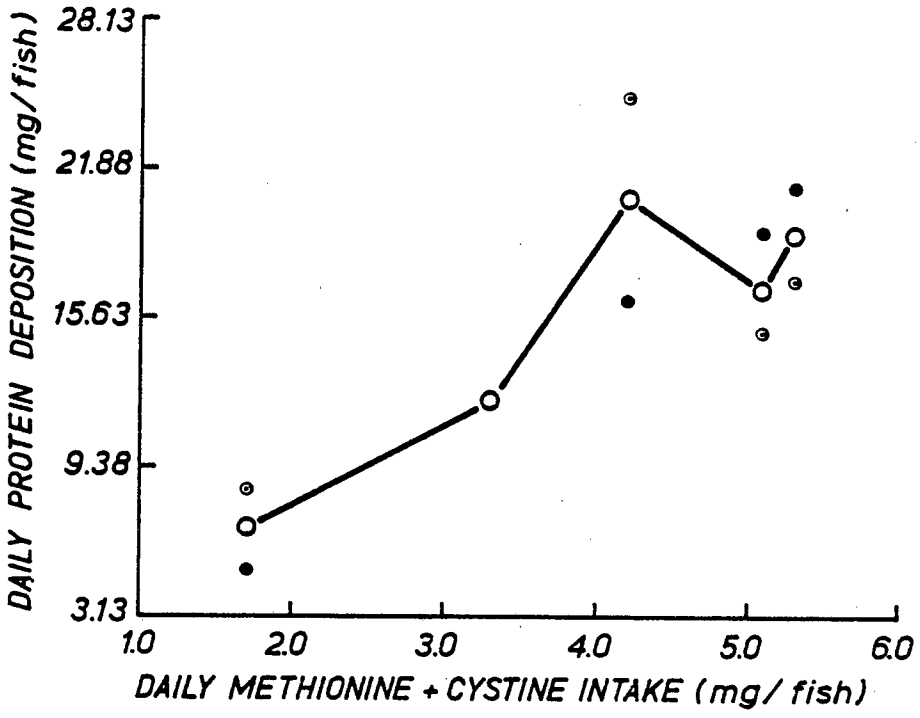


Fig. 23 Exp. 7 Daily methionine + cystine intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 3

weight gain(0.16 g/fish/d) and protein deposition (20.75 mg/fish/d) was observed in the group consuming 4.2 mg methionine/fish/d, which was provided by diet 7M2.

Table 37 compares the results of the terminal composition of fish fed the various dietary methionine concentrations with those of the initial sample. The analysis of variance showed no significant ($P>0.05$) differences in DM, protein, fat, ash and GE content of the experimental fish groups, even when compared with those of the initial sample.

Table 38 shows the effects of feeding different dietary methionine levels on carcass deposition of fat, ash and GE, and the efficiency of protein and GE deposition. A highly significant ($P<0.01$) increase in GE deposition was found in fish given diets 7M2 and 7M3 as compared with those given diets 7M0 and 7M1. The efficiency of GE deposition was significantly ($P<0.01$) higher in fish fed on diets 7M1 and 7M3, but was more significant ($P<0.01$) in fish maintained on diet 7M2 than in those on diet 7M0. The group given diet 7M4, however, showed a significant ($P<0.05$) reduction in the efficiency of GE deposition as compared to those offered diet 7M2.

The results of this experiment indicate that carp are able to utilise L-methionine more efficiently than the DL- form used in Experiment 6. Thus the dietary methionine requirement of carp, at a water temperature of 20°C, is 9.3 g/kg in the presence of 2.4 g cystine/kg DM or 22.6 g in the presence of 5.8 g cystine/kg dietary

TABLE - 37

Experiment 7 - Terminal composition of carp fed on five dietary methionine concentrations at a water temperature of 20°C.

Diet	Dietary methionine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
7M0	6.1	267.3	513.0	295.6	107.4	24.5
7M1	7.6	274.8	506.3	297.8	107.3	24.7
7M2	9.3	280.2	500.3	319.6	107.8	25.1
7M3	10.9	271.1	501.1	307.6	104.4	24.7
7M4	12.5	263.2	528.9	283.0	111.6	24.1
Initial Sample		285.4	502.2	317.8	103.2	25.2
S.E.M.		5.50	15.21	25.67	0.37	2.43
d.f.		6	6	6	6	6

Number of fish per replicate = 13

TABLE - 38

Experiment 7 - Daily carcass deposition of fat, ash and GE of carp fed on diets containing different levels of dietary methionine at a water temperature of 20°C.

Diet	Dietary methionine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition g deposited /g intake	Efficiency of GE deposition kJ deposited /kJ intake
7M0	6.1	-1.10	1.81	0.10	0.12	-0.01
7M1	7.6	3.30	3.19	0.47	0.09	0.08
7M2	9.3	14.20	5.42	1.05	0.14	0.16
7M3	10.9	9.90	4.25	0.86	0.12	0.12
7M4	12.5	0.00	4.09	0.34	0.13	0.05
S.E.M.		4.576	0.656	0.101	0.024	0.022
d.f.		4	4	4	4	4

Number of fish per replicate= 13

protein. It was also found that a daily intake of 4.2 mg sulphur amino acids/fish would promote optimum growth and maximum utilisation of protein.

Experiment 8 :

Since the main objective of this experiment was to estimate the requirement of carp for dietary methionine at a higher temperature ($25^{o}C$), the same isomer (DL-) of methionine used in Experiment 6 at $20^{o}C$ was employed as a supplement.

The growth performance of carp maintained at $25^{o}C$ and fed on diets containing five dietary methionine concentrations (6.1, 7.7, 9.3, 11.0 and 12.6 g/kg represented by 8M0, 8M1, 8M2, 8M3 and 8M4 respectively) is shown in Table 39. A significant ($P<0.05$) increase in weight gain and DM intake was observed within the group given diet 8M3 containing 11.0 g methionine/kg DM. Fish fed on diets containing higher methionine concentrations did not show any further increases ($P>0.05$) in weight gain or DM intake. The efficiency of food conversion was not significantly ($P>0.05$) improved by increasing the dietary methionine concentrations, though there was a gradual improvement with increasing dietary methionine levels up to 11.0 g/kg, after which the response levelled off.

The growth rate of the various fish groups in relation to daily sulphur amino acids intake is illustrated in Fig. 24. A gradual increase in weight gain up to 0.51 g/fish/d was observed as the

TABLE - 39

Experiment 8 - Effect of varying dietary methionine levels on the growth rate, DM intake and efficiency of food utilization of carp maintained at a water temperature of 25°C.

Diet[a,b]	Dietary methionine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
8M0	6.1	0.39	0.61	0.64
8M1	7.7	0.41	0.59	0.71
8M2	9.3	0.51	0.68	0.76
8M3	11.0	0.61	0.75	0.81
8M4	12.6	0.48	0.60	0.80
S.E.M.		0.022	0.021	0.029
d.f.		4	4	4

[a] [b] methionine supplements added in form of the DL-racemic mixture containing 2.4 g cystine/kg DM

Number of fish per replicate = 10

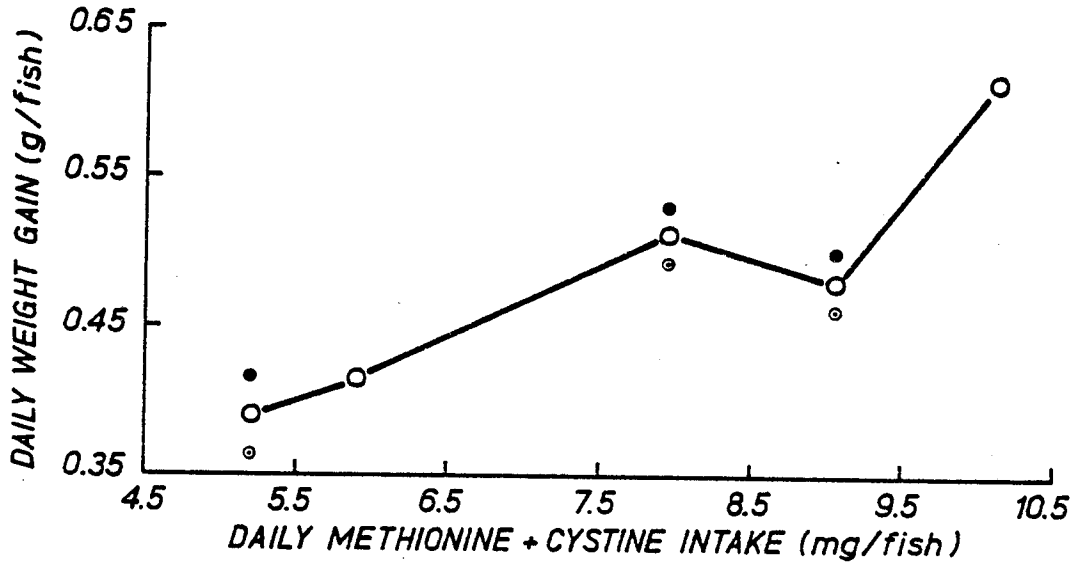


Fig. 24 Exp. 8 Daily weight gain (g/fish) and daily methionine + cystine intake (mg/fish) of young carp maintained at a water temperature of 25°C. O, represents the mean of replicate 1 (⊙) and 2 (●). Methionine supplements were added as racemic mixture (DL-).
Number of fish per replicate = 10

intake of sulphur amino acids increased up to 7.78 mg/fish/d. The fish which consumed 8.78 mg sulphur amino acids/fish/d, provided by diet 8M4, showed a marked decrease in weight gain (0.48 g/fish/d). However, this reduction in weight gain was reversed in the group consuming 10.1 mg sulphur amino acids/fish/d which was provided by diet 8M3 containing less dietary methionine than diet M4. In Fig. 25, the daily protein deposition of fish given the various dietary methionine concentrations is plotted against daily sulphur amino acids intake. The protein deposition increased gradually, in the same manner shown with weight gain, as the intake of sulphur amino acids increased up to 7.78 mg/fish/d. After this level, a marked reduction was observed and this was followed by a sudden recovery at intake level of 10.5 mg sulphur amino acid/fish/d. As mentioned earlier with weight gain, a reduction in protein deposition was observed in the group consuming 8.78 mg sulphur amino acids/fish/d provided by diet 8M4 which contained the highest dietary methionine concentration.

The terminal composition of fish fed the various dietary methionine concentrations in comparison with that of the initial sample is shown in Table 40. No significant ($P>0.05$) differences were found in DM, protein, fat, ash and GE contents of the various fish groups, even when compared with those of the initial sample.

The data on carcass deposition of fat, ash and GE deposition, and the efficiency of protein and GE deposition are presented in Table 41. No significant ($P>0.05$) increase was found in any of these parameters when a statistical comparison was made between all

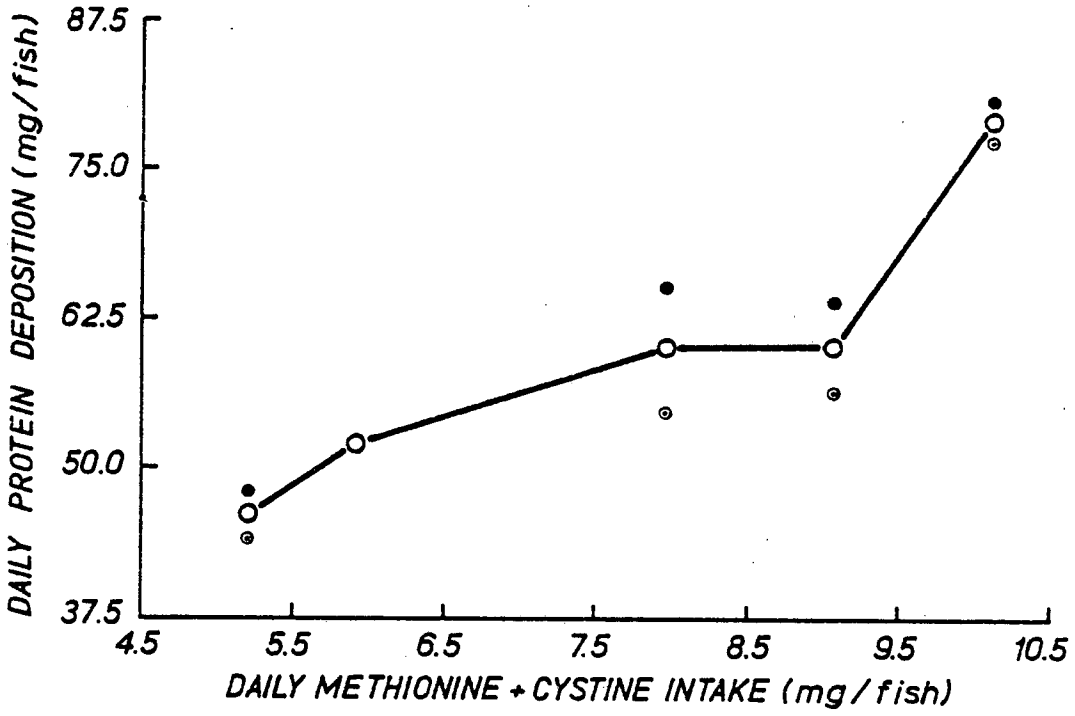


Fig. 25 Exp. 8 Daily methionine + cystine intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 25°C. O, represents the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 10

TABLE - 40

Experiment 8 - Terminal composition of carp fed five dietary methionine levels at a water temperature of 25°C.

Diet	Dietary methionine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
8M0	6.1	258.3	519.1	294.6	113.1	24.8
8M1	7.7	258.7	527.9	279.0	110.0	24.4
8M2	9.3	254.7	519.6	269.4	103.7	24.6
8M3	11.0	252.8	540.1	280.8	107.2	24.1
8M4	12.6	249.4	548.3	255.8	107.5	24.3
Initial Sample		269.4	508.2	299.4	116.7	24.3
S.E.M.		5.38	7.07	14.41	4.52	0.33
d.f.		6	6	6	6	6

Number of fish per replicate = 10

TABLE - 41

Experiment 8 - Carcass deposition of fat, ash and GE in carp fed diets containing different levels of methionine at a water temperature of 25°C.

Diet	Dietary methionine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
8M0	6.1	20.00	11.21	1.62	0.20	0.14
8M1	7.7	17.50	11.11	2.02	0.23	0.19
8M2	9.3	20.30	11.30	2.56	0.23	0.21
8M3	11.0	30.40	14.97	2.98	0.27	0.21
8M4	12.6	12.50	11.06	2.15	0.26	0.20
S.E.M.		6.040	1.183	0.342	0.012	0.033
d.f.		4	4	4	4	4

Number of fish per replicate = 10

the various fish groups. However, optimum performance in respect of these criteria was observed within the group given diet 8M3.

The results of this experiment suggest that the dietary methionine requirement of carp, at a water temperature of 25°C, is 11.0 g/kg in the presence of 2.4 g cystine/kg DM or 28.4 g/kg dietary protein in the presence of 6.2 g cystine/kg dietary protein. The dietary methionine requirement of carp obtained in the present study (at 25°C) is, therefore, similar to that reported at a water temperature of 20°C (experiment 6). A daily intake of sulphur amino acids of 10.1 mg/fish was considered to be adequate for maximum growth and carcass deposition of protein.

Experiment 9 :

Table 42 summarises the effects of feeding three dietary methionine levels [4.5 (9M0), 7.8 (9M1) and 11.0 (9M2) g/kg] and four dietary cystine concentrations [1.1 (C0), 2.2 (C1), 3.3 (C2) and 4.4 (C3) g/kg], in factorial combination, on the growth performance of carp. The growth rate of carp increased significantly ($P<0.05$) with methionine additions. Fish groups fed on the diets containing 7.8 or 11.0 g methionine/kg DM grew significantly ($P<0.05$) better than those on diets containing 4.5 g methionine/kg DM. However, the dietary cystine, and the combined methionine and cystine additions had no signficant ($P>0.05$) effect on the daily weight gain of carp.

The DM intake of the groups given diets containing 7.8 or 11.0

TABLE - 42

Experiment 9 - Effect of varying dietary methionine and cystine levels in factorial combination on weight gain, DM intake and efficiency of food conversion of carp maintained at a water temperature of 25°.

Diet[a,b]	Dietary methionine level g/kg DM	Dietary cystine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
9M0C0	4.5	1.1	0.05	0.37	0.14
9M0C1	4.5	2.2	0.07	0.40	0.19
9M0C2	4.5	3.3	0.09	0.43	0.21
9M0C3	4.5	4.3	0.09	0.43	0.20
9M1C0	7.8	1.1	0.13	0.46	0.28
9M1C1	7.8	2.2	0.14	0.53	0.27
9M1C2	7.8	3.3	0.20	0.63	0.32
9M1C3	7.8	4.3	0.18	0.54	0.33
9M2C0	11.0	1.1	0.13	0.51	0.26
9M2C1	11.0	2.2	0.23	0.65	0.36
9M2C2	11.0	3.3	0.20	0.63	0.33
9M2C3	11.0	4.3	0.15	0.60	0.26
S.E.M.					
	Methionine		0.012	0.011	0.020
	Cystine		0.014	0.012	0.023
	Methionine x Cystine		0.024	0.020	0.040
d.f.					
	Methionine		2	2	2
	Cystine		3	3	3
	Methionine x Cystine		6	6	6

[a] methionine supplements added as the DL-racemic mixture
[b] cystine supplements added as the L-isomer

g methionine/kg was significantly ($P<0.05$) increased when compared with these on diets containing 4.5 g methionine g/kg DM. Generally, the additions of cystine at any dietary methionine level had a significant ($P<0.05$) effect on the DM intake of carp. The DM intake of fish fed on diets containing 3.3 g cystine/kg was significantly ($P<0.01$) higher than those on diets containing 1.1 g cystine/kg DM. A significant ($P<0.05$) increase in DM intake was also observed within the groups offered the diets containing 2.2 or 4.3 g cystine/kg DM when compared with those given diets containing 1.1 g cystine/kg. The combined methionine and cystine additions caused a significant ($P<0.05$) increase in the DM intake of carp. A highly significant ($P<0.01$) increase in DM intake was observed within the groups offered the diets 9M1C2 and 9M2C1 as compared with those given the diets 9M1C0 and 9M2C0 respectively. The DM intake of the groups given diets 9M0C2 and 9M0C3 also increased significantly ($P<0.01$) when compared with that of the group offered diet 9M0C0. Similar findings were obtained within the groups fed on diets 9M1C1 and 9M1C3 as compared with those on 9M1C0, within 9M1C2 as compared with those on 9M1C1 and 9M2C2, and 9M2C3 as compared with those on 9M2C0. But fish fed on diet 9M2C3 consumed significantly ($P<0.05$) more DM than those on diet 9M2C1. However, the DM intake of fish receiving diet 9M1C3 decreased significantly ($P<0.05$) when compared with that of fish given diet 9M1C2.

The efficiency of food utilisation did not signficantly ($P>0.05$) improve by the addition of methionine or cystine separately or in combination. However, optimum efficiency of food conversion was observed within the groups fed on diets 9M0C3

(0.20), 9M1C2 (0.32) and 9M2C1 (0.36) for the three tested levels of dietary methionine concentrations, 4.5, 7.8 and 11.0 g/kg DM respectively.

When daily weight gains of the various fish groups were plotted against the daily intake of sulphur amino acids, three response curves, corresponding to the three dietary methionine levels used, were obtained (Fig. 26). At a dietary methionine level of 4.5 g/kg DM, an increase in weight gain up to 0.09 g/fish/d occurred as the intake of sulphur amino acids increased up to 1.85 mg/fish/d. Further increases in sulphur amino acids intake led to growth retardation. At a dietary methionine level of 7.8 g/kg, a gradual increase in daily weight gain up to 0.2 g/fish/d occurred as the intake of sulphur amino acids increased up to 3.9 mg/fish, after which a marginal growth depression was observed. At a dietary methionine level of 11.0 g/kg, a sudden increase in daily weight gain was observed as the intake of sulphur amino acids increased up to 4.8 mg/fish/d. After this level, excessive intake of sulphur amino acids caused progressive growth retardation. Similar trends (Fig.27) were observed when the daily protein deposition of each fish group was plotted against daily sulphur amino acids intake. Maximum protein deposition of 39.06 mg/fish/d was observed within the group consuming 4.9 mg sulphur amino acids/fish/d, which was provided by diet 9M2C1.

Table 43 shows the results of the body composition of carp fed the various experimental diets in comparison to that of the initial sample. Additions of methionine, cystine or combined methionine

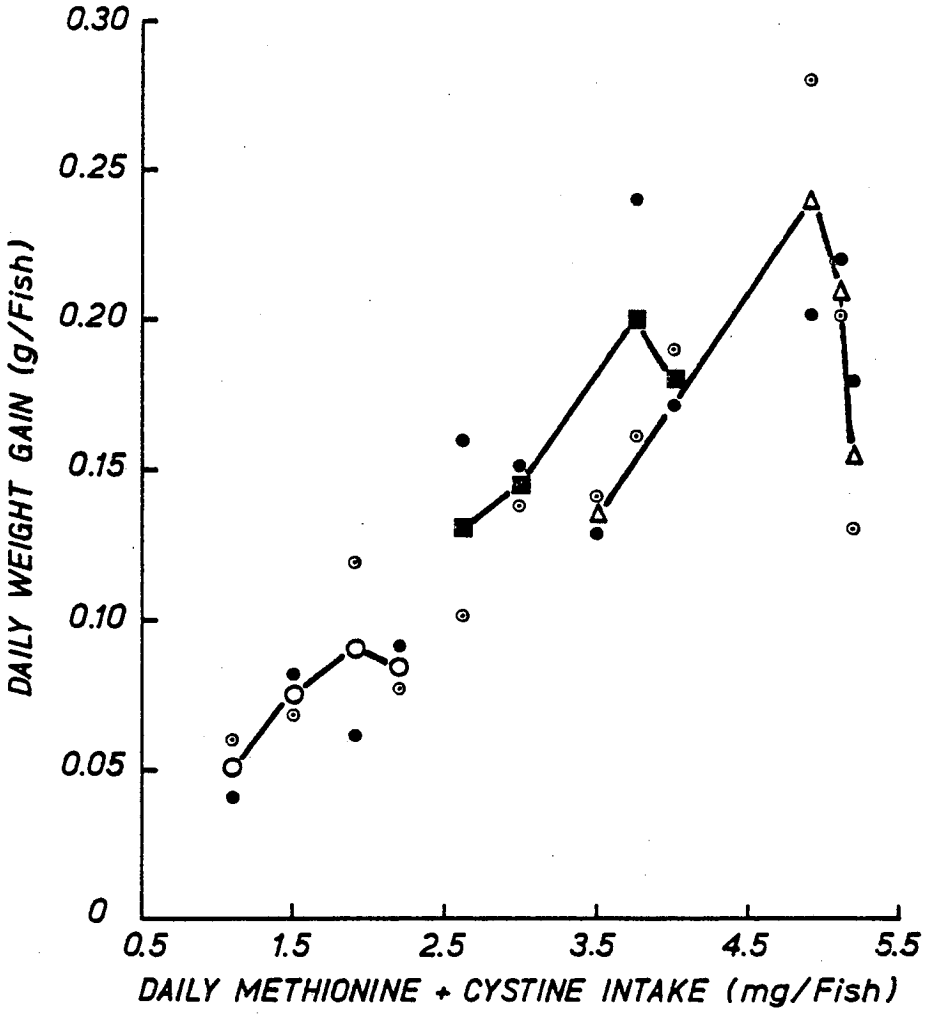


Fig. 26 Exp. 9 Daily weight gain (g/fish) and daily sulphur amino acid intake (mg/fish) of young carp fed three methionine levels of 4.5 (○), 7.7 (■) and 11.0 (△) g/kg DM and four levels of cystine in factorial combinations at a water temperature of 25°C. ○, ■, △, represents the mean of replicate 1 (⊙) and 2 (●) for the three methionine levels respectively. The DL-form of methionine was used as a supplement.
Number of fish per replicate = 12

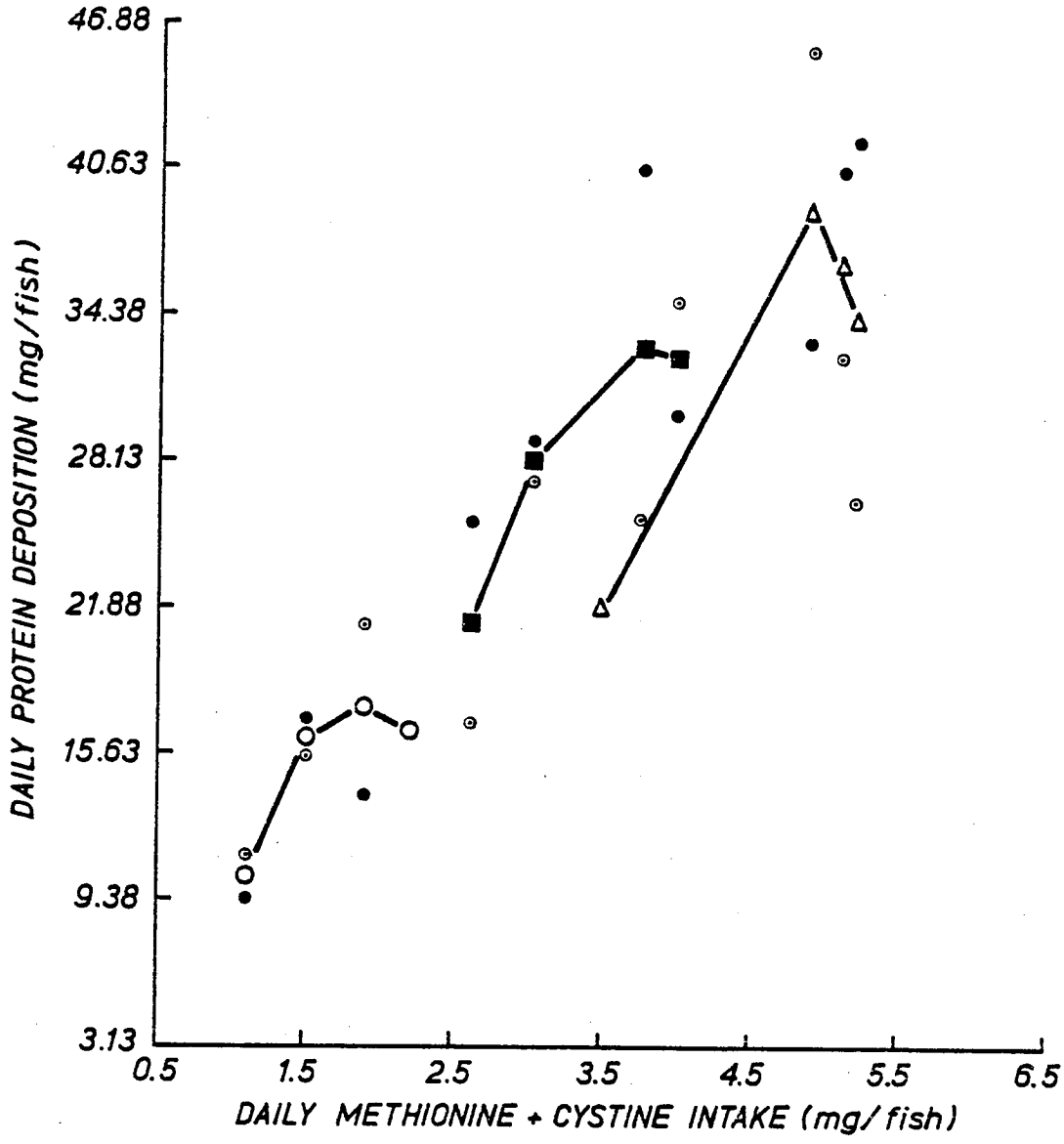


Fig. 27 Exp. 9 Daily protein deposition (mg/fish) and daily methionine + cystine intake of young carp fed three levels of methionine and four levels of cystine in factorial combinations at a water temperature of 20°. O, ■, Δ, represents the mean of replicate 1 (⊙) and 2 (●) for the experimental diets containing 4.5 (O), 7.8 (■) and 11.0 (Δ) g methionine/kg DM. Number of fish per replicate = 12

TABLE - 43

Experiment 9 - Terminal composition in terms of dry matter, protein, fat, ash and GE of carp fed on diets containig different levels of methionine and cystine in factorial combination at a water temperature of 25°C.

Diet	Dietary methionine level g/kg DM	Dietary cystine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
9M0C0	4.5	1.1	224.8	639.4	163.0	143.6	22.4
9M0C1	4.5	2.2	226.9	653.1	175.0	141.3	22.3
9M0C2	4.5	3.3	223.9	654.3	171.2	141.7	22.3
9M0C3	4.5	4.3	226.8	635.6	176.4	150.2	22.2
9M1C0	7.8	1.1	229.7	620.1	202.0	133.0	22.9
9M1C1	7.8	2.2	233.8	645.3	187.6	133.7	22.5
9M1C2	7.8	3.3	233.9	622.3	207.8	128.4	22.9
9M1C3	7.8	4.3	232.6	643.6	189.6	132.3	22.9
9M2C0	11.0	1.1	224.5	637.7	178.0	137.2	22.8
9M2C1	11.0	2.2	231.8	633.0	193.4	130.4	22.8
9M2C2	11.0	3.3	233.9	638.7	194.8	128.7	22.9
9M2C3	11.0	4.3	246.8	652.1	195.4	127.6	23.2
Initial Sample (I.S.)			218.4	617.1	188.8	136.3	23.0
S.E.M.							
I.S. x Met			3.352	11.488	9.477	2.437	0.158
I.S. x Cys			3.465	11.868	9.787	2.517	0.183
I.S. x Met x Cys			4.243	14.533	11.987	3.083	0.316
d.f.							
I.S. x Met			2	2	2	2	2
I.S. x Cys			3	3	3	3	3
I.S. x Met x Cys			6	6	6	6	6

Number of fish per replicate = 12

and cystine did not significantly ($P>0.05$) increase the DM, protein, fat, ash and GE content of carp when compared with that of the initial sample. Moreover, a statistical comparison among the various experimental fish groups did not reveal any significant ($P>0.05$) differences in carcass composition.

The data presented in Table 44 show the effects of feeding the various levels of dietary methionine, cystine and combined methionine and cystine additions on carcass deposition of fat, ash and GE, and the efficiency of protein and GE deposition. No significant ($P>0.05$) increases were found in fat and ash deposition, and in the efficiency of protein and GE deposition at all the dietary methionine and/or cystine concentrations. The GE deposition increased signficantly ($P<0.05$) with an increase in the dietary methionine level from 4.5 to 7.8 or to 11.0 g/kg. However, the combined addition of methionine and cystine or addition of cystine alone did not significantly ($P>0.05$) increase the GE deposition of carp.

The results of this experiment indicate that the dietary methionine requirement of carp is 7.8 g/kg in the presence of 3.3 g cystine/kg, or 11.0 g/kg in the presence of 2.2g cystine/kg DM. When these requirements are expressed in terms of dietary protein, the carp require 18.8 g/kg in the presence of 7.9 g/kg dietary protein or 26.6 g/kg in the presence of 5.3 g/kg dietary protein. These findings indicate, therefore, that the replacement value of cystine for methionine in carp is at least 33%.

TABLE - 44

Experiment 9 - Carcass deposition of fat, ash and GE in carp fed on diets containing different levels of methionine and cystine in factorial combination at a water temperature of 25°C.

Diet	Dietary methionine level g/kg DM	Dietary cystine level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg Deposited /mg intake	Efficiency of GE Deposition kJ deposited /kJ intake
9M0C0	4.5	1.1	0.07	2.50	0.25	0.13	0.07
9M0C1	4.5	2.2	2.46	3.30	0.40	0.19	0.10
9M0C2	4.5	3.3	2.46	3.60	0.44	0.17	0.10
9M0C3	4.5	4.3	3.00	4.70	0.45	0.15	0.10
9M1C0	7.8	1.1	8.08	4.30	0.78	0.16	0.15
9M1C1	7.8	2.2	7.36	5.10	0.86	0.21	0.16
9M1C2	7.8	3.3	12.93	6.00	1.22	0.22	0.19
9M1C3	7.8	4.3	9.12	5.90	1.08	0.27	0.20
9M2C0	11.0	1.1	4.77	4.40	0.71	0.17	0.14
9M2C1	11.0	2.2	11.93	7.30	1.35	0.25	0.20
9M2C2	11.0	3.3	10.98	6.30	1.23	0.26	0.19
9M2C3	11.0	4.3	9.76	5.40	1.13	0.25	0.19
S.E.M.							
	Methionine		1.340	0.395	0.092	0.015	0.016
	Cystine		1.547	0.456	0.106	0.018	0.019
	Methionine x Cystine		2.680	0.790	0.184	0.031	0.033
d.f.							
	Methionine		2	2	2	2	2
	Cystine		3	3	3	3	3
	Methionine x Cystine		6	6	6	6	6

Number of fish per replicate = 12

It should be noticed that at the highest methionine level (11.0 g/kg DM), excessive level of dietary cystine (4.3 g/kg) caused growth retardation and lowered the efficiency of conversion.

At a dietary methionine concentration of 7.8 g/kg, maximum growth rate and protein deposition occurred when the daily intake of sulphur amino acids was 3.9 mg/fish. At a dietary methionine concentration of 11.0 g/kg, the daily intake of sulphur amino acid required to promote maximum growth and protein utilisation was found to be 4.8 mg/fish.

E. Tryptophan Requirements

The aim of both experiments 10 and 11 was to determine the requirements of fingerling carp for dietary tryptophan at 20°C.

1. Experiment 10 :

The growth performance of carp fed on four dietary tryptophan concentrations (1.5, 2.1, 2.6 and 3.2 g/kg, represented by diets 10T0, 10T1, 10T2 and 10T3 respectively) is shown in Table 45. An addition of 0.5 g tryptophan/kg (diet 10T1) to the basal diet (10T0) did not increase the daily weight gain significantly ($P>0.05$). The addition of 1.0 g tryptophan/kg (diet 10T2) to the basal diet resulted in a significant ($P<0.05$) increase in the growth rate (0.11 g/fish/d) of carp. A further addition of 1.5 g tryptophan/kg (diet 10T3) to the basal diet caused marked ($P<0.05$) growth retardation (0.07 g/fish/d).

TABLE - 45

Experiment 10 - Effect of varying dietary tryptophan levels on the growth rate, DM intake and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet	Dietary trypto-phan level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
10T0	1.5	0.05	0.31	0.17
10T1	2.1	0.06	0.27	0.20
10T2	2.6	0.11	0.35	0.32
10T3	3.2	0.07	0.23	0.30
S.E.M.		0.011	0.022	0.054
d.f.		3	3	3

Number of fish per replicate = 14

The DM intake also increased in the same manner as that observed with weight gain, although this increase was not significant ($P>0.05$). Maximum DM intake of 0.35 g/fish/d occurred within the group maintained on diet 10T2 containing 2.6 g tryptophan/kg DM, after which an increase in the dietary tryptophan concentrations (diet 10T3) caused a marked reduction in DM intake. Maximum efficiency of food conversion was also achieved with the group fed on diet 10T2.

The vitamin and mineral mixture used in this experiment was different from that used in the other experiments, and the growth rate attained by carp in this experiment was slightly lower than expected. Since Experiment 11 was planned to confirm the results achieved in Experiment 10, with reference to the body composition, no attempt was made to study the effect of the different concentrations of dietary tryptophan on the carcass composition of carp.

The results obtained from this experiment suggest that the tryptophan requirement of carp is 2.6 g/kg DM or 6.1 g/kg dietary protein. A dietary tryptophan concentration of 3.2 g/kg caused a marked reduction in the growth performance of carp.

2. Experiment 11 :

The data on the growth rate and efficiency of food conversion of carp fed on four dietary tryptophan levels (1.5, 2.1, 2.6, and 3.2 g/kg, represented by diets 11T0, 11T1, 11T2, and 11T3

respectively) is summarised in Table 46. The enhanced response to supplementation of the basal diet (11T0) with tryptophan confirmed the inadequacy of the diet with respect to this amino acid. A significant ($P<0.05$) increase in weight gain (0.07 to 0.19 g/fish/d) was obtained with diet 11T2 which contained 2.6 g tryptophan/kg DM. An excess level of dietary tryptophan (diet 11T3) retarded the growth and reduced the efficiency of food utilisation. The dry matter intake, however, increased progressively as dietary tryptophan levels were increased, but these differences were not significant ($P>0.05$).

The growth rate and protein deposition in relation to daily tryptophan intake of fish fed on the various experimental diets are shown in Figs. 28 and 29 respectively. Maximum weight gain of 0.19 g/fish/d and protein deposition of 36.25 mg/fish/d occurred within the group that consumed 1.7 mg tryptophan/fish/d which was supplied by diet 11T2.

The effect of dietary tryptophan on body composition of carp is summarised in Table 47. No significant ($P>0.05$) differences were observed in DM, protein, fat, ash and GE content of the various experimental fish groups and those of the initial sample. The analysis of variance also showed no significant ($P>0.05$) differences in DM, protein, fat and GE content in the various fish groups. The ash content of fish fed diet 11M1 was significantly ($P<0.05$) higher than those fed on the other diets.

As is obvious from the data presented in Table 48, optimum

TABLE - 46

Experiment 11 - Effect of varying dietary tryptophan levels on the growth rate and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet	Dietary trypto-phan level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain /g DM intake
11T0	1.5	0.07	0.45	0.16
11T1	2.1	0.11	0.56	0.19
11T2	2.6	0.19	0.63	0.31
11T3	3.2	0.17	0.63	0.28
S.E.M.		0.019	0.028	0.032
d.f.		3	3	3

Number of fish per replicate = 10

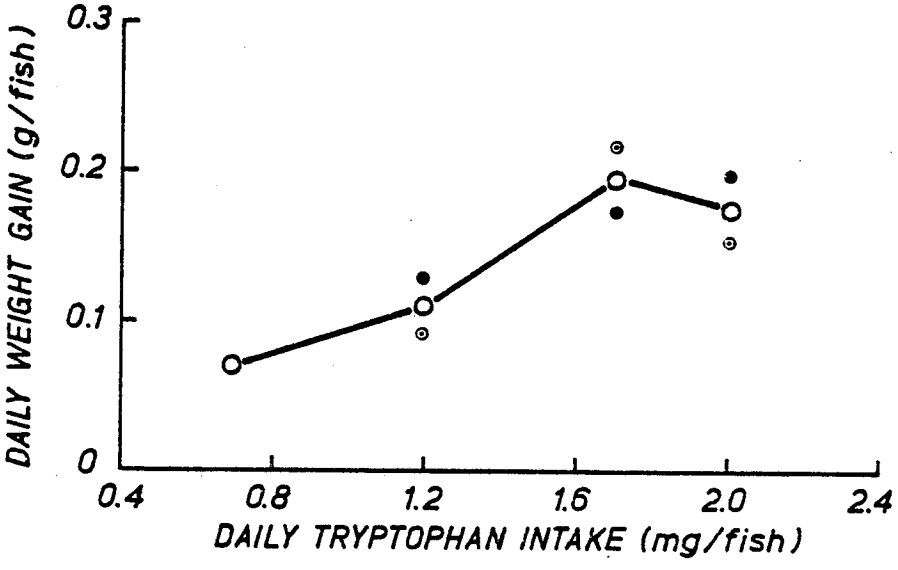


Fig. 28 Daily weight gain (g/fish) and daily tryptophan intake (mg/fish) of young carp maintained at a water temperature of 20°C. **O**, represents the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 10

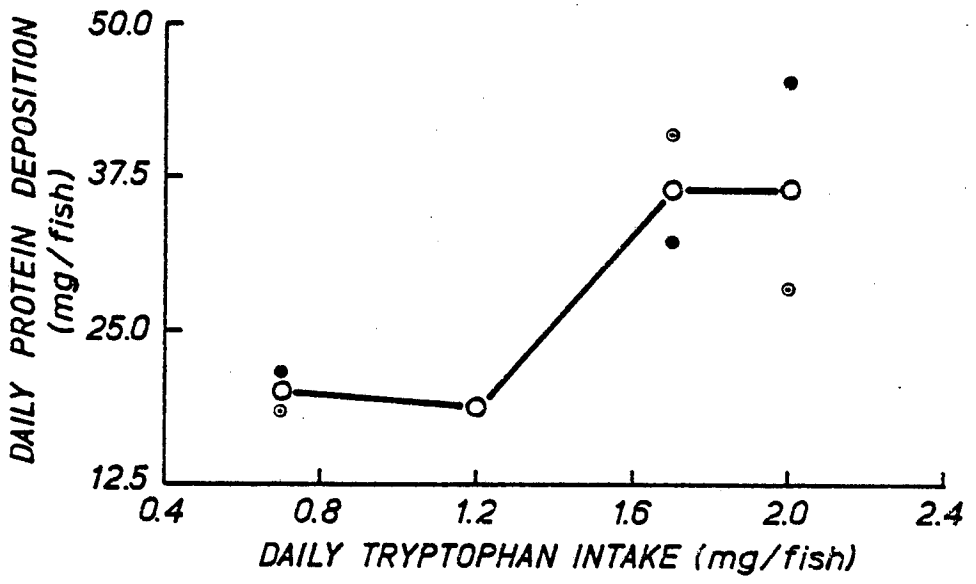


Fig. 29 Exp. 11 Daily tryptophan intake (mg/fish) and daily protein deposition (mg/fish) of young carp maintained at a water temperature of 20°C. O, represents the mean of replicate 1 (⊙) and 2 (●).
Number of fish per replicate = 10

TABLE - 47

Experiment 11 - Terminal composition in terms of dry matter, protein, fat, and ash of carp fed on diets containing different levels of dietary tryptophan at a water temperature of 20°C.

Diet	Dietary tryptophan level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
11T0	1.5	257.2	585.4	210.2	128.5	22.8
11T1	2.1	260.4	555.9	208.6	132.8	22.4
11T2	2.6	266.4	558.8	218.8	130.9	23.0
11T3	3.2	268.7	563.6	234.4	122.5	23.0
Initial Sample		227.9	621.3	122.8	152.2	20.6
S.E.M.	S.E.M.	5.22	13.42	8.25	3.89	0.31
d.f.	d.f.	5	5	5	5	5

Number of fish per replicate = 10

TABLE - 48

Experiment 11 - Carcass deposition of fat, ash and GE in carp fed on diets containing graded levels of dietary tryptophan at a water temperature of 20°C.

Diet	Dietary trypto-phan level g/kg DM	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
11T0	1.5	30.90	0.64	1.65	0.11	0.20
11T1	2.1	32.90	3.56	1.80	0.08	0.18
11T2	2.6	42.60	6.94	2.67	0.15	0.23
11T3	3.2	46.80	3.94	2.61	0.15	0.23
S.E.M.		3.836	1.183	0.332	0.021	0.025
d.f.		3	3	3	3	3

Number of fish per replicate = 10

efficiency of protein and GE deposition was observed in the group given the diet 11T2 containing 2.6 g tryptophan/kg DM, although the results were not significant (P>0.05). The carcass deposition of fat increased progressively with raised dietary tryptophan levels ,but without showing any statistical significance (P>0.05). The deposition of ash in the fish given diet 11T1 was almost 6 times (3.56 mg/fish/d) that of the group offered the basal diet (0.64 mg/fish/d). This increase in ash deposition continued as the dietary tryptophan level (11T2) was increased. As can be seen from Table 48, the deposition of ash in fish given diet 11T2 was about 2 times that observed in the group maintained on diet 11T1. However, fish fed on the diet containing a higher concentration of tryptophan (11T3) showed a fall in ash deposition to 3.94 mg/fish/d. Analysis of variance showed no significance (P>0.05) in any of these results.

From the results obtained in this experiment, the tryptophan requirement of carp is 2.6 g/kg DM or 6.7 g/kg dietary protein. An excessive level of dietary tryptophan (3.18 g/kg) induced an inhibitory effect on the growth performance of carp. These findings confirm the results reported in experiment 10. When the requirement of carp was expressed in terms of intake, it was found that a daily intake of 1.7 mg tryptophan/fish was adequate to support maximum growth and protein utilisation.

F. Histidine Requirements

Experiment 12 :

This experiment was planned to assess the requirement of fingerling carp for dietary histidine at 20°C.

Table 49 summarises the growth performance of carp fed five dietary histidine concentrations (5.2, 5.7, 6.3, 6.8 and 7.4 g/kg, represented by diets 12H0, 12H1, 12H2, 12H3 and 12H4 respectively). No significant ($P>0.05$) differences were found in weight gain, DM intake and the efficiency of food conversion of fish fed the basal diet (12H0) and those on diets containing supplementary histidine. A significant ($P<0.05$) reduction in weight gain occurred within the group maintained on diet 12H4, which contained the highest dietary histidine concentration. At this level, however, the efficiency of food conversion was also adversely affected, though this observation was not significant ($P>0.05$).

The body composition of fish fed various dietary histidine concentrations in comparison with that of the initial sample is shown in Table 50. No significant ($P>0.05$) differences were found in protein, fat, ash and GE contents of carp fed the various experimental diets, nor when compared with those of the initial sample.

Table 51 compares the results of carcass deposition of protein, fat, ash and GE deposition, and the efficiency of protein and GE deposition of fish fed the various levels of dietary histidine. Increasing the dietary histidine concentrations did not significantly ($P>0.05$) affect the deposition of ash and the efficiency of protein and GE deposition of carp. However, fish fed

TABLE - 49

Experiment 12 - Effect of varying dietary histidine levels on the growth performance of carp maintained at a water temperature of 20°C.

Diet	Dietary histidine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
12H0	5.2	0.11	0.31	0.37
12H1	5.7	0.10	0.30	0.31
12H2	6.3	0.11	0.31	0.36
12H3	6.8	0.11	0.30	0.37
12H4	7.4	0.05	0.27	0.19
S.E.M.		0.010	0.011	0.071
d.f.		4	4	4

Number of fish per replicate = 15

TABLE - 50

Experiment 12 - Terminal composition of carp fed diets containing five levels of dietary histidine at a water temperature of 20°C.

Diet	Dietary histidine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
12H0	5.2	276.7	504.8	311.4	104.2	25.0
12H1	5.7	269.8	528.8	275.2	112.1	24.4
12H2	6.3	269.3	522.3	280.2	110.4	24.3
12H3	6.8	268.1	525.9	293.2	111.8	24.5
12H4	7.4	259.0	532.2	266.2	118.0	24.8
Initial Sample		285.4	502.2	317.8	103.2	25.2
S.E.M.		6.82	12.54	20.82	2.48	0.60
d.f.		6	6	6	6	6

Number of fish per replicate = 15

TABLE - 51

Experiment 12 - Carcass deposition of protein, fat, ash and GE in carp fed on diets containing five levels of dietary histidine at a water temperature of 20°C.

Diet	Dietary histidine level g/kg DM	Daily protein deposited mg/fish	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
12H0	5.2	13.50	7.60	2.86	0.63	0.13	0.12
12H1	5.7	30.00	6.70	6.80	1.08	0.27	0.21
12H2	6.3	14.44	1.00	3.43	0.41	0.12	0.08
12H3	6.8	14.75	2.90	3.65	0.42	0.12	0.08
12H4	7.4	4.69	-7.40	2.19	-0.06	0.04	-0.02
S.E.M.		7.810	5.021	1.596	0.282	0.077	0.058
d.f.		4	4	4	4	4	4

Number of fish per replicate = 15

on diet 12H4 showed a marked ($P<0.05$) reduction in the deposition of protein, fat and GE.

These results indicate that the dietary levels of histidine under the present experimental conditions were not limiting. It was also observed that a dietary histidine level of 7.4 g/kg caused a marked growth depression of carp. Thus the requirement for dietary histidine is less than 5.2 g/kg or less than 15.0/kg dietary protein. Under the present experimental conditions, a daily intake of 1.6 mg/fish was considered to be adequate to support maximum growth and optimum utilisation of protein.

G. Threonine Requirements

Experiment 13 :

The purpose of this experiment was to investigate the requirement of fingerling carp for dietary threonine at a water temperature of 20°C.

The growth performance of carp fed five levels of dietary threonine (8.4, 10.0, 11.7, 13.3 and 15.0 g/kg, represented by diets 13Th0, 13Th1, 13Th2, 13Th3 and 13Th4 respectively) is summarised in Table 52. No significant ($P<0.05$) differences in weight gain, DM intake or efficiency of food conversion were observed among the various experimental fish groups.

The terminal composition of fish fed the different

TABLE - 52

Experiment 13 - Effect of varying dietary threonine levels on daily weight gain, DM intake and efficiency of food conversion of carp maintained at a water temperature of 20°C.

Diet	Dietary threonine level g/kg DM	Daily weight gain g/fish	Daily DM intake g/fish	Efficiency of food conversion g gain/g DM intake
13Th0	8.4	0.12	0.37	0.32
13Th1	10.0	0.14	0.39	0.34
13Th2	11.7	0.12	0.39	0.31
13Th3	13.3	0.13	0.38	0.34
13Th4	15.0	0.16	0.42	0.38
S.E.M.		0.014	0.020	0.029
d.f.		4	4	4

Number of fish per replicate = 10

concentrations of dietary threonine is compared with that of the initial sample (Table 53). A highly significant ($P<0.01$) increase in the protein content was observed in fish fed the various dietary threonine levels (except those fed diet 13Th3) as compared with that of the initial sample. The fish fed on diet 13Th3, however, showed a highly significant ($P<0.01$) reduction in protein content when compared with the other experimental groups. The fish given diets 13Th0, 13Th1 and 13Th2 showed a significant ($P<0.05$) reduction in GE contents as compared with those of the initial sample. No significant ($P>0.05$) differences were found in GE contents of fish given the diets 13Th3 and 13Th4 when compared with those of the initial sample. A statistical comparison among the various experimental fish groups revealed a significant ($P<0.05$) increase in GE content of fish offered diet 13Th3 as compared with those maintained on diets 13Th0, 13Th1 and 13Th2. The DM, fat and ash contents were not significantly ($P>0.05$) affected by the various levels of dietary threonine.

Table 54 gives the data on carcass deposition of protein, fat, ash and GE, and the efficiency of protein and GE deposition in fish fed the different levels of dietary threonine. The carcass deposition of GE and the efficiency of GE deposition were significantly ($P<0.05$) increased in fish fed on diet 13Th3 as compared with those maintained on diet 13Th0, 13Th1 and 13Th2. Fish given diet 13Th3 showed a significant ($P<0.05$) increase in GE deposition and the efficiency of GE deposition when compared with those fed on diet 13Th2.

TABLE - 53

Experiment 13 - Terminal composition of carp fed on five dietary threonine concentrations at a water temperature of 20°C.

Diet	Dietary threonine level g/kg DM	DM g/kg tissue	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE kJ/kg DM
13Th0	8.4	282.0	528.8	303.0	110.0	24.4
13Th1	10.0	280.8	532.3	299.4	110.4	24.6
13Th2	11.7	278.6	532.9	289.8	107.9	24.2
13Th3	13.3	292.5	492.7	322.8	106.3	25.3
13Th4	15.0	282.0	536.1	295.0	104.7	24.7
Initial Sample		285.4	502.2	317.8	103.2	25.24
S.E.M.		3.39	4.32	6.06	2.40	0.17
d.f.		6	6	6	6	6

Number of fish per replicate = 10

TABLE - 54

Experiment 13 - Carcass deposition of protein, fat, ash and GE in carp fed on diets containing varying levels of dietary threonine at a water temperature of 20°C.

Diet	Dietary threonine level g/kg DM	Daily protein deposited mg/fish	Daily fat deposited mg/fish	Daily ash deposited mg/fish	Daily GE deposited kJ/fish	Efficiency of protein deposition mg deposited /mg intake	Efficiency of GE deposition kJ deposited /kJ intake
13Th0	8.4	25.88	7.50	5.65	0.70	0.18	0.11
13Th1	10.0	24.56	1.40	5.31	0.64	0.17	0.08
13Th2	11.7	22.69	10.50	4.20	0.44	0.15	0.06
13Th3	13.3	19.13	15.20	5.33	1.13	0.13	0.17
13Th4	15.0	31.00	7.00	4.76	0.93	0.19	0.12
S.E.M.		2.709	2.596	0.916	0.081	0.017	0.014
d.f.		4	4	4	4	4	4

Number of fish per replicate = 10

These results suggest that the tested levels of dietary threonine were not limiting. Thus the dietary threonine requirement of carp is less than 8.4 g/kg DM or 21.2 g/kg dietary protein. Under the present experimental conditions, the carp required a daily intake of 3.0 mg threonine/fish to promote a maximum growth rate and to induce optimum deposition of protein. This intake of threonine was provided by the basal diet.

IV. General Discussion

As viewed in terms of dietary concentrations, the requirements of fish for protein and amino acids are thought to be influenced by several considerations (Sections I-C-3 and I-E-3). Among these are species, age and size of fish, nutritional factors and environmental temperature. When requirements are expressed in terms of daily intake, similarities in amino acid utilisation by endothermic animals have been demonstrated under various experimental conditions (D'Mello, 1975; D'Mello and Emmans, 1975; D'Mello, 1976; 1978; 1982). However, as compared with that knowledge available in the case of terrestrial vertebrates, data concerning protein and amino acid requirements of fish, as a function of intake, is scarce.

In recent years, considerable emphasis has been placed on the need to express amino acid requirements in terms of daily intake, as this methodology takes into account those factors which influence requirements through changes in voluntary food ingestion (D'Mello, 1975; D'Mello and Emmans, 1975; D'Mello, 1976; 1978; 1982; Boorman, 1980). In the current investigation, the amino acid requirements of carp were estimated in terms of intake as well as in terms of dietary concentrations. Such an approach allows some speculation as to the similarities in amino acid requirements which may exist between different species of fish.

As dietary concentrations, the protein requirements of growing animals decrease with increasing age (Halver, 1970; Graber et

al,1971; Satia, 1974; Halver 1976a,b; NAS/NRC, 1977a; Balarin and Haller, 1982). Since the sum of the individual amino acid requirement totals the protein requirements, it might be expected that amino acid needs would also decrease with increasing age.

Most studies on the requirements of fish for protein (DeLong et al, 1958; Dupree and Sneed, 1966; Ogino and Saito, 1970; Dabrowski, 1977; Mazid et al, 1979) and amino acids (Halver et al, 1958; 1959; Klein and Halver, 1970; Nose, 1978; Robinson et al, 1980; 1981; Jackson and Capper, 1982) have been carried out with specimens ranging in size from fry to fingerling. A survey of the literature shows that little information is available on the protein and amino acid requirements of juvenile and mature fish, although several authors (Halver, 1970; Satia, 1974; Halver, 1976a,b; NAS/NRC, 1977a; Balarin and Haller, 1982) have pointed out that requirement decreases with increasing age. Furthermore, no data has yet appeared on the intake of protein or amino acid necessary to promote their optimum growth. Similarities in the requirements of fry, fingerling, juvenile and mature fish cannot be demonstrated without the provision of relevant data as to the intake of protein and amino acids.

As has been outlined in Section I-E-1, a;b;c, several methods are available for determining the amino acid requirements of an animal. Among these are the graded supplementation and diet dilution techniques. Although the former method has not proved to be inferior to the latter in the assessment of amino acid requirements of non-ruminant animals (D'Mello, 1982), the graded supplementation

technique was employed in the current investigations, as it has frequently been applied in nutritional studies with fish.

The amino acid requirements of channel catfish (see for example, Wilson et al, 1977, 1978, 1980) and carp (Nose, 1978) were determined using the graded supplementation technique and purified, amino acid, test diets similar to those employed by Halver (1957b) in the case of chinook salmon. Jackson and Capper (1982) examined the amino acid requirements of tilapia (Sarotherodon mossambicus) using semi-purified diets. However, with a view to formulating practical diets for fish, these authors indicated that the amino acid requirements of fish determined by semi-purified diets might be more applicable than those estimated with purified diets. The use of the graded supplementation technique incorporating semi-purified diets was used to determine the amino acid requirements of carp, as this allowed comparisons to be made between this and other species of fish in their amino acid requirements, as dietary concentrations and as daily intake.

Two published reports (Nose, 1978; Ogino, 1980) are available on the amino acid requirements of carp. It should be noted that the experimental conditions used in the present studies are considerably different from those of Nose (1978) and completely different from those of Ogino (1980). In the current studies, semi-purified diets were formulated from intact protein sources instead of from the synthetic amino acid mixture employed by Nose (1978). The carp used in the present studies (Appendix C) were much larger than the specimens employed by Nose (1978). The

estimated amino acid requirements derived from the current investigations were based on the growth rate of fish, amino acid intake and protein utilisation, in addition to several other criteria which were all subjected to statistical analysis. The assessment of amino acid requirements reported by Nose (1978) was based solely on the measurement of growth rate, and his findings were not subjected to any statistical analyses. In the present studies, the requirements for lysine and sulphur-containing amino acids were investigated at water temperatures of 20 and 25°C. In addition, the replacement value of cystine for methionine was assessed by the addition of three methionine and four cystine concentrations in factorial combination; whereas Nose (1978) assessed the requirement for methionine without and with an excessive dietary concentration of cystine. As the carcass composition procedure employed by Ogino (1980) seems to ignore the amino acid requirements for maintenance and other functions (Walton et al, 1982), the values given by Ogino (1980) were not used for comparative purposes in the current studies.

A. Interpretation of Results

The importance of maintaining the dietary protein level above the minimum protein requirement when determining the quantitative requirement for amino acids, has been emphasised by Bressani and Mertz (1958) and by Mertz (1969). On the other hand, there is growing evidence that the requirement (expressed as g/kg diet) of fish for dietary protein and amino acids decreases as the fish advance in age. For these reasons, it was considered that the

first step in the current studies should be to determine protein requirements. The amino acid requirements, as dietary concentrations and daily intake, could then be assessed in the certain knowledge that protein was not limiting maximum performance.

1. Protein Requirements

Carp were found to be unable to grow on diets in which the protein was derived solely from crystalline amino acids (Aoe et al, 1970). In several other instances, carp exhibited a very low efficiency of food utilisation when maintained on such purified diets (Nose, 1974, 1978). However, Ogino and Saito (1970) were able to determine the quantitative protein requirements of carp fry using casein as the sole source of dietary protein. They concluded that carp fry required 380 g protein/kg diet. The determined protein requirement of fingerling carp, obtained from the present study (Table 18), is 389 g/kg. This estimate parallels that reported by Ogino and Saito (1970) and is also similar to the values published for most other species of fish (Table 4). It confirms, moreover, the conclusions of Mertz (1969) that the protein requirement of fish is 2-4 times higher than that of endothermic animals. Due to the absence of any discrepancy between the value reported by Ogino and Saito (1970) for carp fry and the determined figure in the present study for fingerling carp, it is possible that the requirements of carp are similar at both stages of life.

Studies on the requirements of fish have shown a linear increase in their growth rate and/or in carcass deposition of protein as a result of increasing dietary protein up to concentrations of between 350-450 g/kg (Dupree and Sneed, 1966; Nose and Arai, 1973; Lim et al, 1979). In the present studies, the growth rate and carcass deposition of protein increased linearly as the intake of protein was augmented. The sudden surge observed in the growth rate of groups that consumed daily amounts of 280 mg protein/fish (Fig. 10), provided by the highest level of protein used (Table 18), could be related to the improvement in the ratio of ME to protein in the diet given to this group as compared to the other diets. Another explanation is that the amino acid content of the same diet was adequate to furnish the dietary needs of carp for indispensable amino acids.

Several biological procedures were used to determine the nutritive value of food proteins in studies involving the quantitative and qualitative protein requirements of fish. Among these were determination of the efficiency of protein deposition, NPU and BV. As indicated in section I-B-2, estimation of the efficiency of protein deposition is, at present, the most useful and most practical method of evaluating food protein for fish (Higuera et al, 1977; Cowey and Sargent, 1979).

Higuera et al (1977) assessed the nutritive value of a normal commercial trout diet by determining the efficiency of protein deposition, NPU and BV in rainbow trout. It was found that all three values obtained were in close agreement. The efficiency of

protein deposition (Table 18) obtained from the present study (0.15-0.23) is of the same order as that (0.23) reported by Higuera et al (1977) and parallels the optimal values (0.20-0.25) quoted by Cowey (1980). The main objective of this investigation, however, was to assess the quantitative protein requirement by using semi-purified diets.

Studies on the protein requirements of young carp (Ogino and Saito, 1970) and young tilapia (Mazid et al, 1979) have shown that the PER decreased linearly as a result of increased dietary protein levels. Similarly, in the present study, a gradual decrease was noted in the efficiency of protein deposition, from 0.23 to 0.15, as a result of increasing dietary protein from a level of 181 to 282 g/kg, after which it remained constant. The highest efficiency of protein deposition observed with fingerling carp fed on the lowest dietary protein level may reflect a very low maintenance requirement for protein. Similar conclusions were previously drawn by Ogino and Saito (1970) with young carp, and by Mazid et al (1977) with tilapia. At higher dietary protein concentrations, however, a part of the dietary protein might be used as a source of energy. Whether fish species are able to utilise high proportions of dietary carbohydrates without diverting amino acids from protein synthesis, needs to be investigated with relevant data on food intake and carcass deposition of protein.

In the current investigation (Experiment 1), carp were able to utilise high proportions (up to 377 g/kg) of starch at a dietary protein level of 181 g/kg (Table 18). The lowest dietary protein

concentration, coupled with this level of starch, may be responsible for the significant increase in carcass content of fat (Table 19), as compared with that of an initial sample taken at the beginning of the experiment. The gradual reduction in carcass content of fat observed with carp fed on the various experimental diets could reflect the gradual increases in the dietary content of protein. Ogino et al (1976) showed that at low dietary protein levels, rainbow trout utilised dietary fat more effectively than carp, but that the latter were capable of utilising dietary carbohydrates more effectively than the former. These authors claimed that the requirement of rainbow trout for dietary protein was influenced by the source of dietary energy. When the data of Ogino et al (1976) was plotted against protein intake, a single response curve was obtained (Section I-C-3-d, Fig. 3), showing clearly that protein utilisation is not influenced by the source of dietary energy. An analogous situation exists in the case of terrestrial animals. Thus it is well recognised that poultry, for example, can derive their requirements for energy equally from fat or carbohydrates without affecting protein utilisation (Renner, 1964; ARC, 1975).

In the current studies, the progressive reduction in carcass deposition of fat (Table 20) observed when increasing the levels of dietary protein, reflects the trend discovered with young carp (Ogino and Saito, 1970; Meske and Pfeffer, 1978c), plaice (Cowey et al, 1972) and eel (Nose and Arai, 1973). This effect, however, is in opposition to the trend observed with the grass carp (Dabrowski, 1977).

It should be noted that a protein intake of 281.19 mg/fish/d induced maximum carcass deposition of protein, and relatively low carcass deposition of fat in carp. This intake was supplied by a dietary protein concentration of 389 g/kg.

2. Protein and Lysine Interactions

Studies with endothermic, terrestrial animals (Grau, 1948; Grau and Kamei, 1950; Bringer et al, 1950; Bressani and Mertz, 1958; Harper, 1964) have shown that the dietary lysine requirement, expressed as g/kg diet, increased with increasing the protein content of the diet. On the other hand, the addition of excessive levels of lysine to a low-protein diet caused growth depressions (Sauberlich, 1961; Harper et al, 1970). The amino acid profile in the first case remained balanced, as the increases in both dietary lysine and in all the other amino acids, which were the constituents of the protein, was proportional (Harper, 1964; Harper et al, 1970). Hence a normal growth performance was achieved. The reason for the growth depression of the animal in the second case was attributed to an amino acid imbalance and to a disproportionate addition of dietary lysine (Harper, et al, 1970). Additional protein was therefore required to prevent, either partially or completely, the growth depression (Sauberlich, 1961).

In the present study, the estimated dietary lysine requirement of carp was higher when the protein content of the diet was increased (Table 21). These findings are in line with those reported for endothermic animals (Grau, 1948; Grau and Kamei, 1950;

Bringer et al, 1950, Bressani and Mertz, 1958; Harper, 1964). The results obtained from the present investigation also indicate that carp are similar to terrestrial vertebrates in their inability to tolerate excessive levels of lysine (Harper, 1958; Jones et al, 1966; O'Dell and Regan, 1963; D'Mello and Lewis, 1970a).

Terminal body composition (Table 22) and carcass deposition of ash, and the efficiency of protein and GE deposition (Table 23) in carp were not significantly affected by the level of dietary protein or lysine additions. GE deposition is the only criterion which showed a significant improvement as a result of increasing the dietary protein level. The reason for the insignificant results obtained with other parameters could be related mainly to the small number and large variation in the size of fish studied.

At the higher level of protein (389 g/kg diet), it is important to note that increasing the dietary concentrations of lysine resulted in a progressive increase in carcass deposition of fat (Table 23). In order to avoid undesirable carcass deposition of fat, it is necessary to adjust the dietary concentration of lysine against the level of protein. It must be emphasised that further studies are rquired to understand more fully the biochemical role of lysine-protein interaction and its effect on the carcass composition of carp.

3. Lysine Requirements

The dietary lysine requirement of fingerling carp (14.0 g/kg) determined in Experiments 3 and 4 (Tables 24 and 27 respectively) is considerably lower than that reported for young carp (22.0; Nose, 1978), chinook salmon (20.0; Halver et al, 1958) and eel (20.0 g/kg; Arai and Nose, cited by NAS/NRC, 1981), but higher than that estimated for channel catfish (15.0 g/kg; Robinson et al, 1980b). The dietary lysine requirement of carp (Experiment 5) is apparently also higher than that (>13.3 g/kg) reported for tilapia by Jackson and Capper (1982). These findings demonstrate clearly that real differences exist among fish species in their requirements (as g/kg diets) for lysine. These differences could be mainly attributed to the method of expressing requirements and not to the various species or to the different experimental conditions involved.

The results of Experiments 3 and 4 (Tables 24 and 27 respectively) indicate that the dietary lysine requirement of fingerling carp, maintained in recycled or non-recycled water at 20°C, is 14.0 g/kg. This estimate is considerably less than the figure of 18.9 g/kg found in experiment 5 (Table 30), which was conducted at a water temperature of 25°C. These findings are similar to those reported for the chick (March and Biely, 1972) which suggest, superficially at any rate, that the dietary lysine requirement, expressed as g/kg, is dependent upon the environmental temperature. The effect of temperature on lysine requirements deserves further elaboration in view of the comments of D'Mello (1978) on the data published by March and Biely (1972). These authors examined the lysine requirements of chicks at two

environmental temperatures, 20 and 31.1°C. When the responses were considered in relation to dietary concentration of lysine (Fig. 30), two distinct curves were obtained. This result implied a fall in the efficiency of lysine utilisation at the higher temperature. However, when the same data was considered in relation to daily intake of lysine (Fig. 31), it was shown that the utilisation of lysine was not impaired at the higher temperature. Chicks maintained at 31.1°C merely ate less food. A greater dietary concentration of lysine was therefore required to compensate for the reduction in appetite at the higher temperature. The daily intake of lysine required to support the stated rates of growth was found to be similar at both temperatures investigated.

A similar approach to that employed by D'Mello (1978) in the case of chicks was applied to carp, which were maintained at water temperatures of 20° (Experiment 3) and 25°C (Experiment 5). From these experiments, the requirements of carp were considered in terms of dietary lysine concentration (Fig. 32) and daily intake of lysine (Fig. 33). When considered as a function of dietary lysine concentration, two growth response curves were obtained (Fig. 32), which implies a decrease in the efficiency of lysine utilisation at the lower temperature. However, when the same data was plotted against daily intake of lysine, similarities in lysine utilisation were obvious (Fig. 33). For further comparison, values for lysine intakes of carp, obtained from Experiment 4, were also considered (Fig. 34). As can be seen, the pattern of responses to the intake of lysine remained consistent under the various experimental conditions. Thus a daily intake of 10.0 mg lysine/fish would

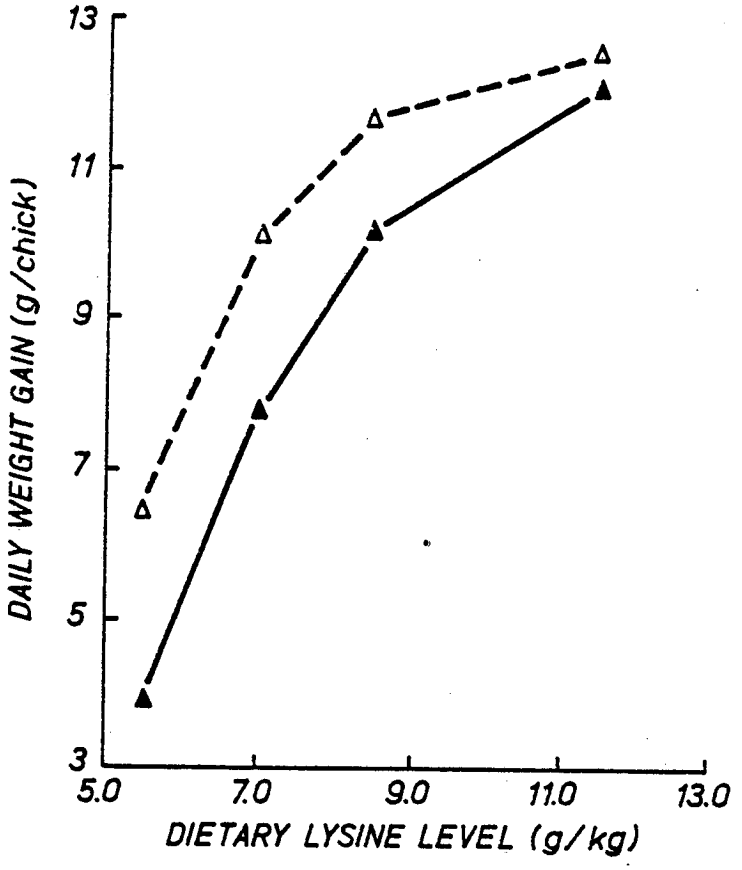


Fig. 30 Daily weight gain (g/chick) of chicks fed different dietary concentrations of lysine and maintained at two environmental temperatures 20 (Δ) and 31.1°C (▲). (After D'Mello, 1978)

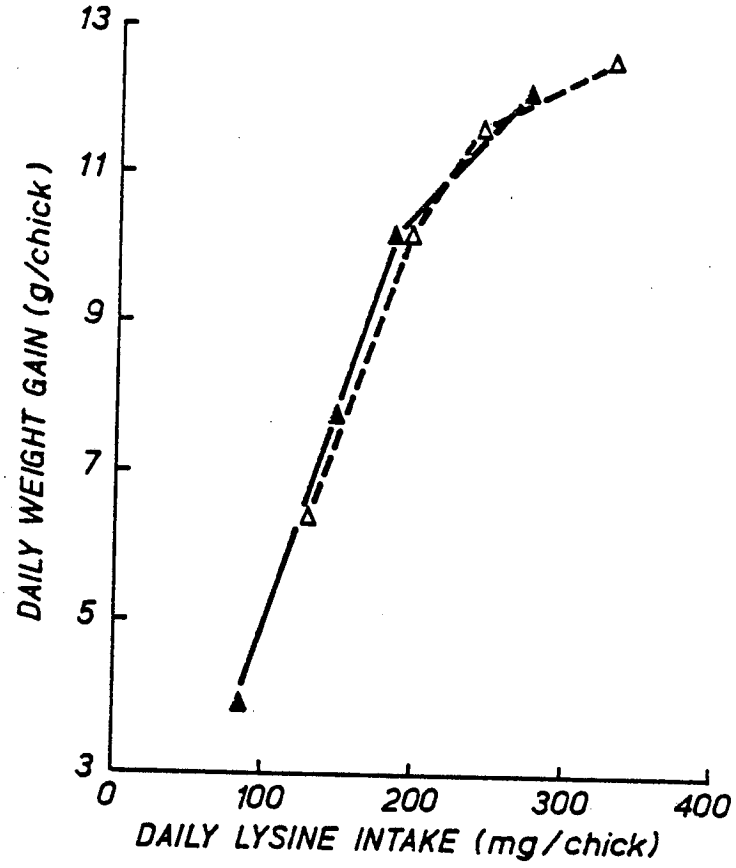


Fig. 31 Daily weight gain (g/chick) and daily lysin intake (mg/chick) of chicks maintained at two environmental temperatures, 20 (Δ) and 31.1°C (▲). (After D'Mello, 1978)

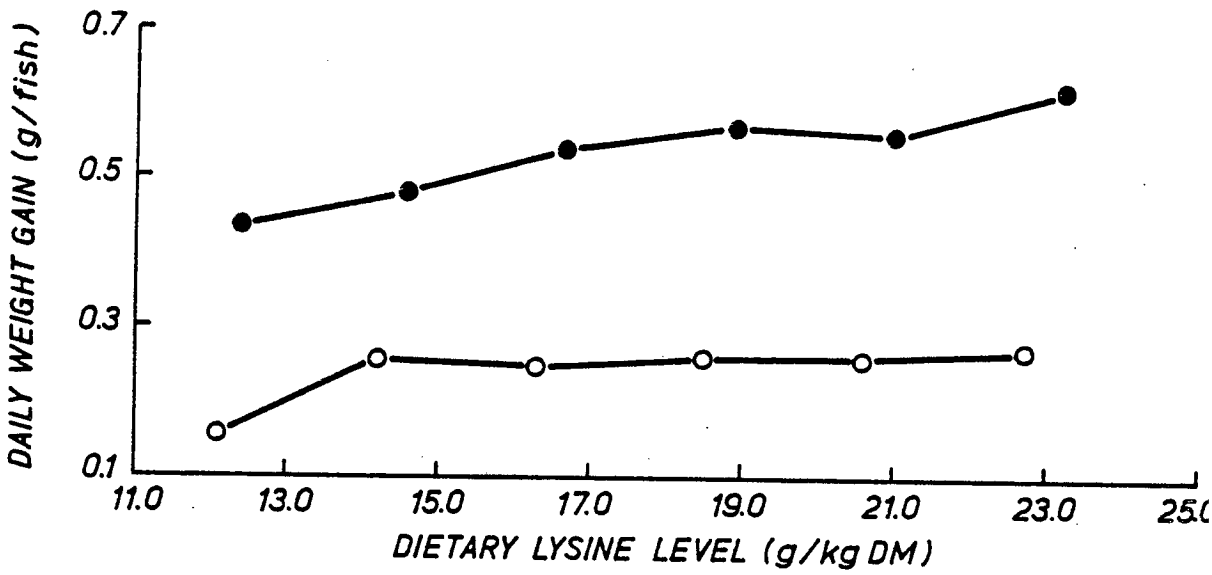


Fig. 32 Daily growth responses (g/fish) of carp fed different concentrations of lysine (g/kg DM) and maintained at two two water temperatures, (O), 20°C, (●) 25°C. (Data from Experiments 3 and 5 respectively)

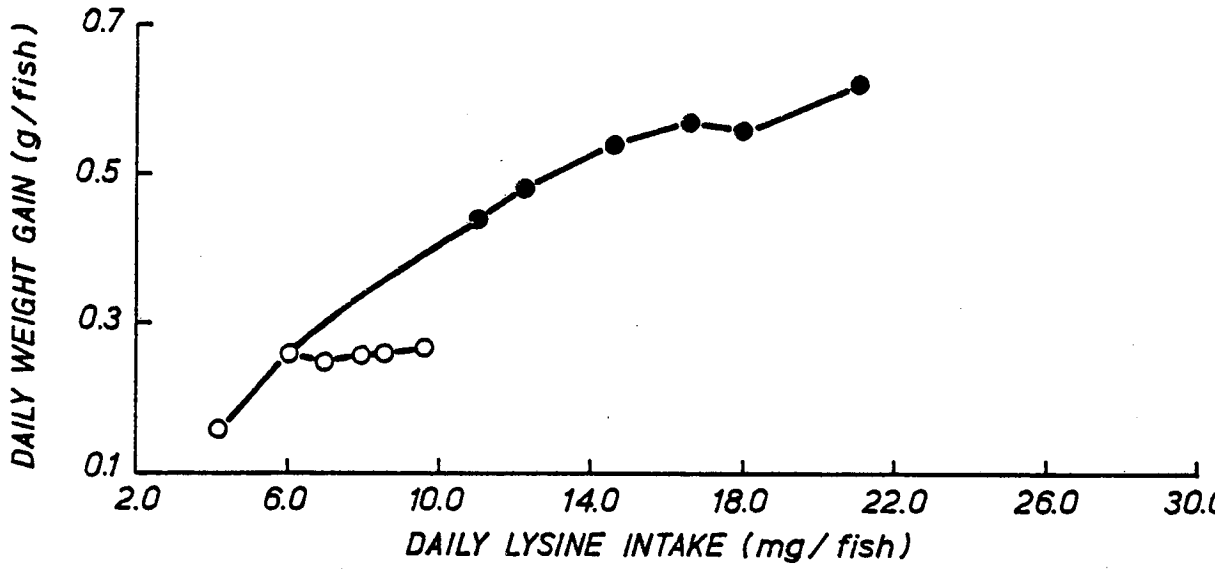


Fig. 33 Daily weight gain (g/fish) and daily lysine intake (mg/fish) of carp maintained at two water temperatures, (○) 20°C and (●) 25°C. (Experiments 3 and 5 respectively)

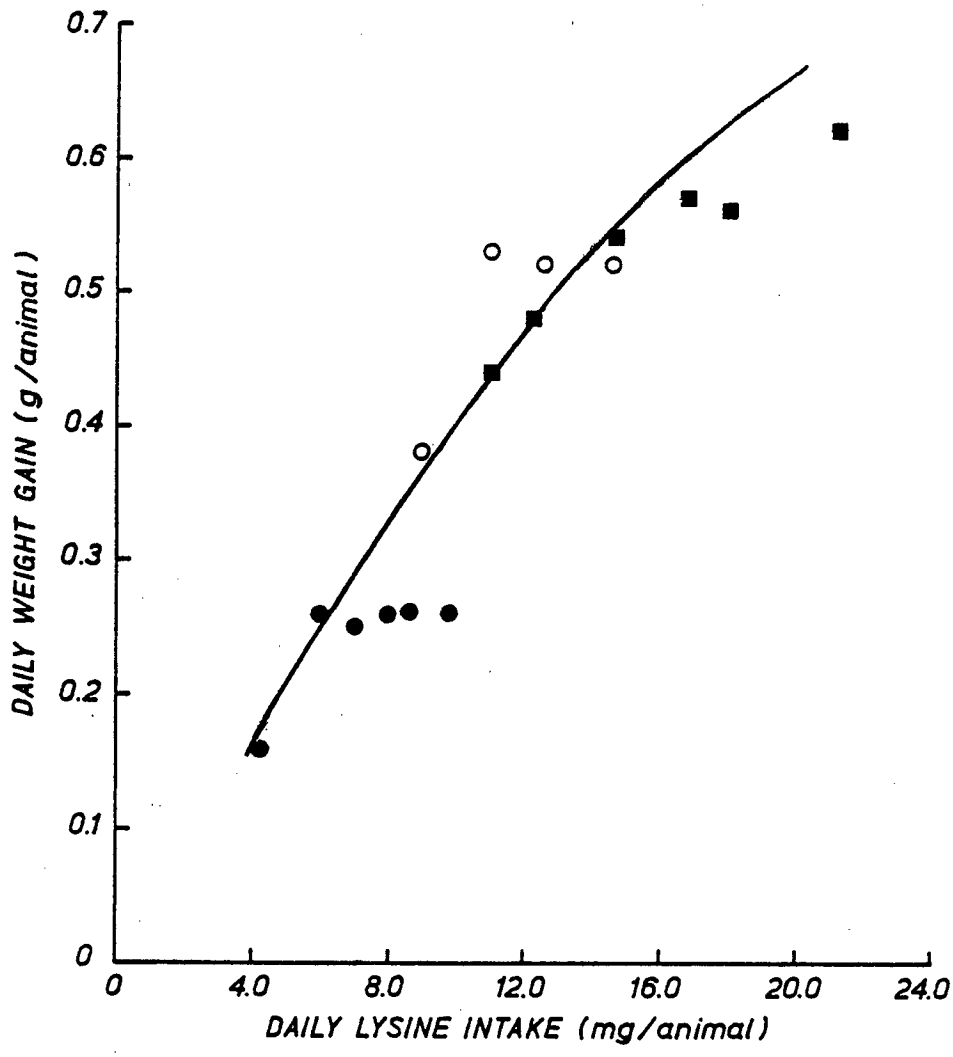


Fig. 34 Growth responses (g/fish/d) of carp, maintained in recycled (●) and non-recycled water (○) at 20°C, and at 25°C in non-recycled water (■), in relation to lysine intake (mg/fish/d). Data from Experiments, 3, 4 and 5 respectively.

promote a similar daily weight gain (0.35 g/fish) in carp maintained at either temperature.

One of the primary objectives of the current experimental programme was to establish some basis for the comparison of amino acid requirements of different species of fish. Such an approach seemed logical in view of the studies of D'Mello (1976), who concluded that the methionine plus cystine requirements for growth were similar for chicks, turkeys and the laboratory rat, providing these requirements were expressed in terms of daily intake, rather than in terms of dietary concentrations. In 1981, NAS/NRC suggested that the expression of amino acid requirements, as a proportion of dietary protein, would provide a useful technique for comparing the amino acid requirements of different species of animals including fish. However, in this thesis, the approach of D'Mello (1976) has been adopted since the NAS/NRC (1981) suggestions ignore the effect of variations in voluntary food intake which may exist among different species of fish. These variations can occur as a result of changes in dietary energy concentration (D'Mello, 1978) and as a result of changes in environmental temperature (Figs. 31-34). Another factor which may determine voluntary food intake is the species of animal (D'Mello, 1978). It is of considerable interest, therefore, to establish whether the reported variations in amino acid requirements of different species of fish could be attributed to some genuine genetic differences in amino acid utilisation or to differences in food intake. Accordingly, the approach devised by D'Mello (1976) with terrestrial vertebrates was employed to study variations in

amino acid requirements of different species of fish.

Lysine was selected for this detailed study since it is easily analysed in normal ingredients and because its biochemical and nutritional fate is largely unaffected by dietary concentrations of other nutrients. The data presented in Fig. 34 (Experiments 3-5) and discussed earlier in this section was plotted in a further graph (Fig. 35) which also incorporates data from published work on other species of fish. This graph (Fig. 35) thus includes data on the growth rates of carp, tilapia (Jackson and Capper, 1982) and channel catfish (Wilson et al, 1977) in relation to daily intake of lysine. In view of the differences in environment, husbandry and feeding techniques employed in the various experiments, it is remarkable that the data for the three species of fish should be so compatible. The single response curve suggests that for a given rate of growth the lysine requirement expressed as daily intake is similar in the three species of fish. This single response curve also indicates that the efficiency of lysine utilisation is similar in the three species. Thus a daily intake of 4 mg lysine/animal would promote a daily weight gain of 0.2 g/animal (Fig. 35). It is concluded, therefore, that genetic factors exert their influence primarily throughout altering food intake and not by influencing the efficiency of lysine utilisation. If lysine utilisation had been different in the three species of fish, this would have manifested itself as three distinct curves in Fig. 35.

In Experiment 3, the significant increase in carcass deposition of fat (Table 26) in fish given lysine concentrations of 16.3, 18.5

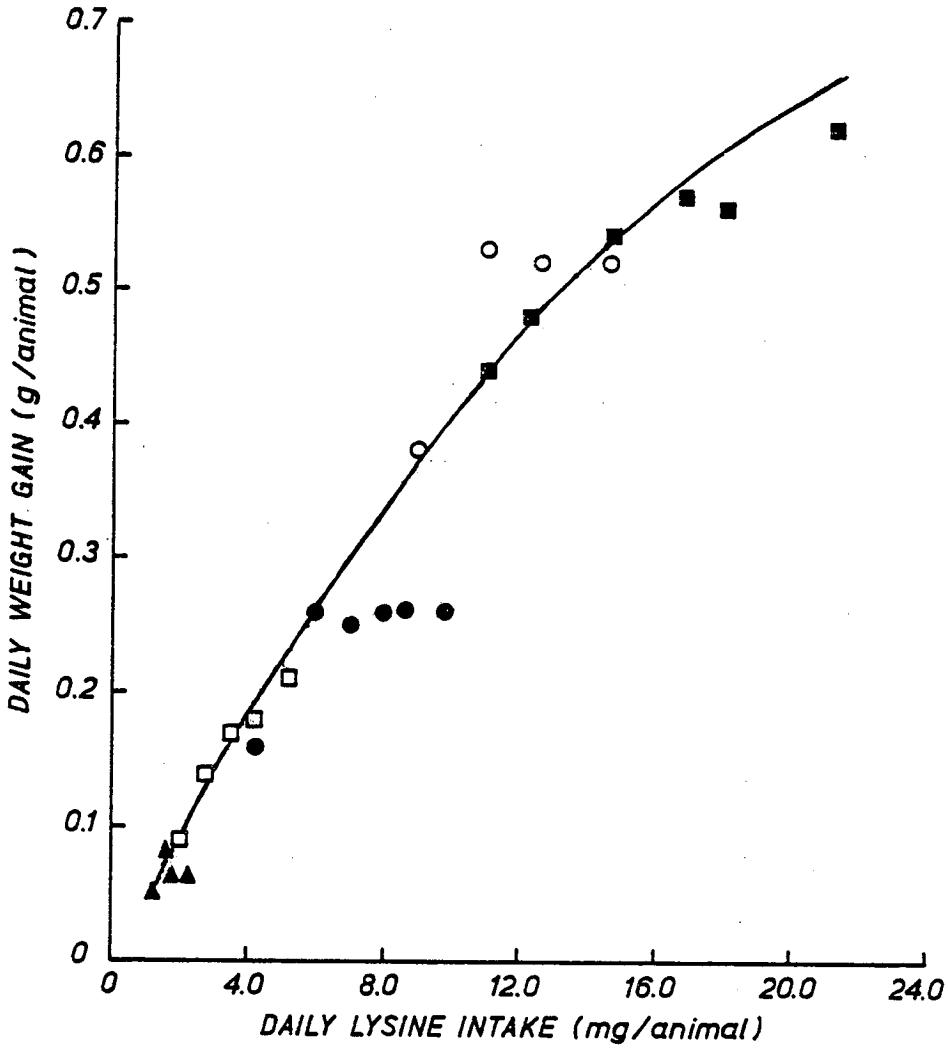


Fig. 35 Growth responses of tilapia, channel catfish and carp in relation to lysine intake (mg/fish/d). Tilapia data (▲, 25°C) from Jackson and Capper (1982); channel catfish data (□, 26.7°C) from Wilson et al (1977); and carp data (●, 20°C; ○, 20°C; ■, 25°C) from Experiments 3, 4 and 5 respectively

and 20.6 g/kg diet indicates that a lysine concentration of 14.0 is also optimal for carcass fat deposition. These observations were in close agreement with those obtained from Experiment 5 conducted at a water temperature of 25°C. A dietary lysine concentration of 14.0 g/kg may therefore be considered adequate, not only for optimal protein utilisation by carp, but also for the prevention of excessive deposition of carcass fat.

Fat retention is assumed to be very high in carp (Meske and Pfeffer, 1978c). As the composition of the product is of considerable interest to the consumer, it seemed proper to examine in detail the effect of dietary lysine on the body composition of fish. It should be noted, however, that excessive concentrations of dietary lysine could result in excessive deposition of carcass fat in carp.

In Experiment 4, the relatively low carcass deposition of protein observed with fish given the highest level of lysine could be attributed to excessive intake of lysine (Fig. 17). A similar response was not elicited when the same level was fed in Experiment 3 (Fig. 15). This test was conducted at a similar water temperature of 20°C using smaller fish than those employed in Experiment 4 (Appendix C). This result may indicate that larger fish are more susceptible to excessive levels of dietary lysine, as the daily intake of lysine of these fish was about twice that of the corresponding group in Experiment 3.

Growth depression (Fig. 18) and a relatively low protein

deposition (Fig. 19) were also observed within the group fed on the highest level of dietary lysine in Experiment 5, conducted at 25°C. The daily lysine intake of this group was about twice that of the corresponding group fed on the same diet maintained at 20°C (Experiment 3). These findings emphasise the need to measure the intake of the amino acid in studies aimed at determining the amino acid requirement of the animal. A more detailed investigation into the adverse effects of an excessive intake of lysine may be justified.

4. Requirements for sulphur-containing amino acids

The requirements of carp for the sulphur amino acids (methionine and cystine) were determined under a variety of environmental and nutritional conditions. The effects of two water temperatures on these requirements were therefore investigated. In addition, the utilisation of DL- and L-isomers of methionine were also examined, as were the effects of varying dietary ratios of methionine to cystine.

a. Studies with supplements of DL-methionine

The results obtained from Experiments 6 and 8 (Tables 33 and 39 respectively) indicate that the methionine and cystine requirements, as g/kg diet, of fingerling carp fed on identical diets supplemented with DL-methionine are similar at the two temperatures investigated (20 and 25°C). As will later be shown in Fig. 36, these conclusions are in direct conflict with earlier

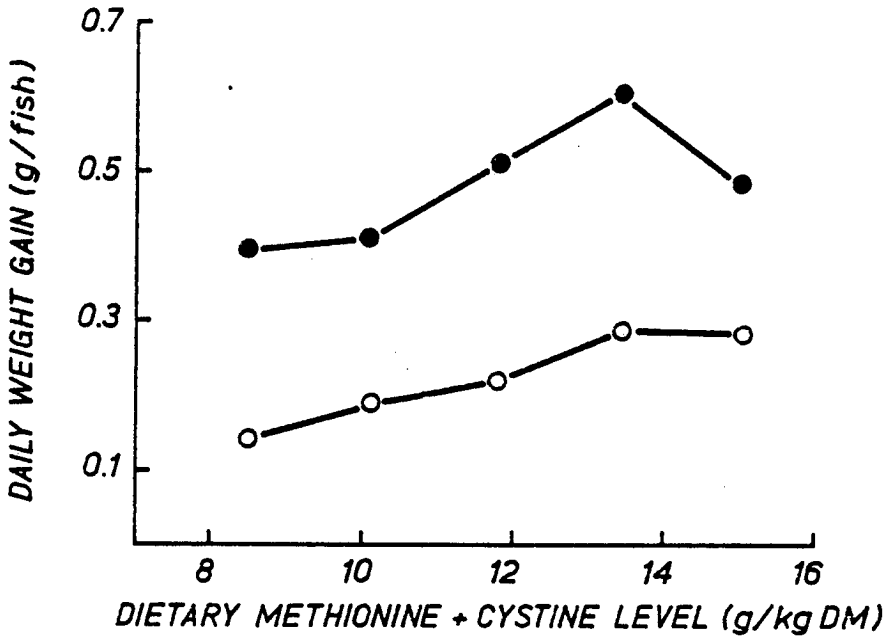


Fig. 36 Daily weight gain (g/fish) of carp fed different levels of dietary methionine + cystine (g/kg) and maintained at two water temperatures, 20°C (O) and 25°C (●). Data from Experiments 6 and 8 respectively

findings (Fig. 32) on the lysine requirements of carp (Experiments 3, 4 and 5) and chicks (Fig. 30; March and Biely, 1972), in that these requirements, expressed in terms of dietary concentrations, rose with increased environmental temperature. The reason for the similarity in methionine and cystine requirements of carp at the two temperatures examined may be related to the methionine-cystine inter-dependence.

Since the basal diets used in both experiments were identical in terms of ingredients, it is therefore possible that the cystine concentration (2.4 g/kg diet) could be over the level required by carp maintained at a water temperature of 20°C. In contrast, the cystine concentration of the same diets used at a water temperature of 25°C becomes limiting at this higher temperature. In other words, a part of the dietary methionine might have been used for cystine synthesis in order to meet the increased demand for the total sulphur amino acid at the higher temperature. However, a dietary methionine concentration of 11.0 g/kg in the presence of 2.4 g cystine/kg, could therefore be considered adequate for carp maintained at a water temperature of 25°C. A slightly lower concentration might be required by carp maintained at a water temperature of 20°C.

The requirement of carp for the sulphur amino acids (11.0 g methionine and 2.4 g cystine/kg diet) as determined in the present study (Experiments 6 and 8), is marginally higher than the value (12.0 g/kg) reported for carp fry by Nose (1978) using only methionine as a sulphur amino acid. However, the value obtained

from Experiments 6 and 8 is considerably lower than the figure of 28 g/kg, also obtained by Nose (1978) using an excessive concentration (20 g/kg) of dietary cystine.

When expressed as g/kg dietary protein, the requirement of fingerling carp for sulphur amino acids (Experiments 6 and 8) is higher than that reported for channel catfish (Harding et al, 1977); rats and pigs (Mertz, 1969) and turkeys (D'Mello, 1976). By contrast, fingerling carp (Experiments 6 and 8) appeared to require about half the amount needed by carp fry (Nose, 1978) and eel (Arai and Nose, unpublished data, in Cowey and Sargent, 1979).

More recently, Jackson and Capper (1982) demonstrated that the requirement of tilapia for sulphur amino acids is less than 32 g/kg dietary protein. This estimate may indicate the similarity between carp (Experiments 6 and 8) and tilapia in their requirements for sulphur amino acids. However, the differences observed in the requirements for dietary sulphur amino acids of the various fish species could be attributed to the fact that no common methodology was applied in the determination of these requirements, particularly in the dietary proportion of methionine, cystine and other sulphur-containing components. In addition, the different isomers of methionine used by the different investigators may account, in part, for these discrepancies.

When the growth responses of carp were considered in terms of dietary concentrations of methionine and cystine (Fig. 36), two distinct response curves were obtained from the two temperatures

tested. However, when these growth rates are plotted against daily intake of the sulphur amino acids, it is seen that the utilisation of methionine and cystine is broadly similar at both temperatures except for the groups consuming 5.2, 5.7 and 9.0 mg sulphur amino acid/fish/d at the higher temperature. The reason for this discrepancy is attributable, in the main, to increased food intake stimulated by raising the temperature. This resulted in excessive intake of imbalanced diets (D'Mello, 1976, 1978), either deficient in methionine or containing excessive amounts of this amino acid. As already indicated, the inter-relationship between methionine and cystine may also account for the slightly worse fit of data in Fig. 37.

The maximum growth rate (Fig. 20) and protein deposition (Fig. 21) obtained at 20°C are much lower than that found in the corresponding fish group maintained at 25°C and given a similar dietary concentration of the sulphur amino acids (Figs. 24 and 25 respectively). The group maintained at 20°C consumed considerably less food. A higher intake of sulphur amino acids was therefore required to induce maximum growth and protein deposition at 25°C. Indeed, the intake of sulphur amino acids of the group exhibiting maximum performance and maintained at the higher temperature, was about twice (Figs. 24 and 25) that of the corresponding group maintained at the lower temperature (Figs. 20 and 21).

As shown earlier in Table 35, maximum carcass deposition of fat (23.43 mg/fish/d) was observed in fish fed adequate dietary concentrations of methionine and cystine (13.4 g/kg). On the other

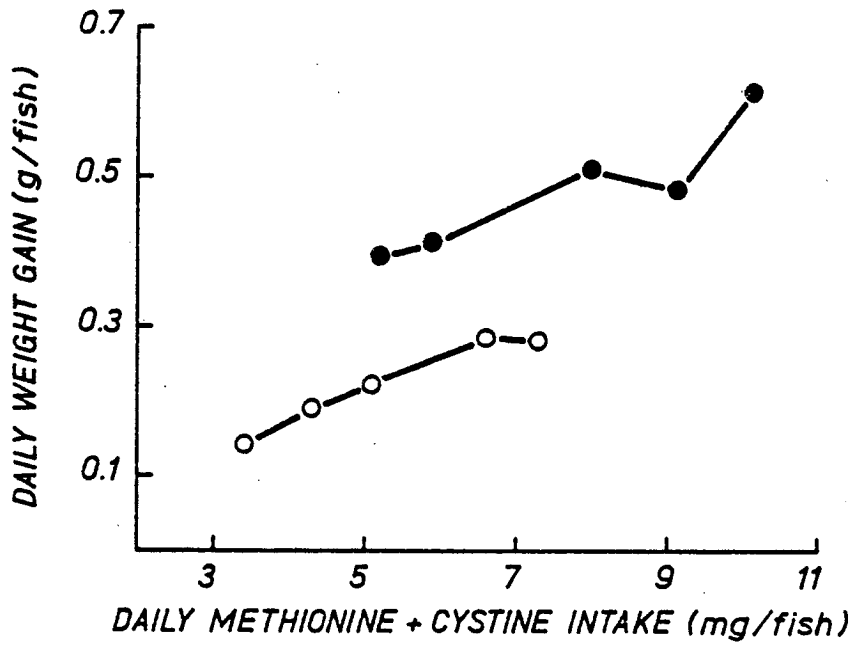


Fig. 37 Daily weight gain (g/fish) and daily methionine + cystine intake (mg/fish) of carp maintained at two water temperatures 20°C (O) and 25°C (●). Data from Experiments 6 and 8 respectively

hand, fish offered a dietary methionine level of 9.4 g/kg exhibited considerably lower carcass deposition of fat (2.90 mg/fish/d). These findings are in close agreement with those recorded in the subsequent experiments, 7 and 8, though the differences were not statistically significant. These observations, however, suggest the importance of maintaining dietary methionine at a marginally lower level than the suggested estimate for maximum growth performance, in order to avoid undesirable accumulation of fat in carp tissue.

b. L-methionine supplementation

D'Mello and Lewis (1978) reviewed evidence that rats and chicks are similar in their ability to utilise the L-form of methionine more effectively than the racemic mixture. Recently, Kaushik and Luquet (1980) reported that rainbow trout utilised the L-form of methionine more easily than the DL-form, a finding which is consistent with the conclusion of D'Mello and Lewis (1978). In contrast, channel catfish (Robinson et al, 1978) showed similar utilisation of both isomers.

The estimated dietary sulphur amino acid requirements (Table 36) of carp obtained by using the L- form of methionine as a supplement (Experiment 7), are markedly lower (11.8 g/kg) than that (13.4 g/kg) determined with the DL-isomer (Experiment 6). The results of Experiment 7 would therefore indicate that carp are similar to the rat and chick (D'Mello and Lewis, 1978) and rainbow trout (Kaushik and Luquet, 1980) in utilising the L-form of

methionine more effectively than the DL-isomer. One of the practical repercussions of these results is that supplemental levels of methionine can probably be reduced for carp by using the L-form, although it should be appreciated that for economic reasons, the widespread commercial supplementation of diets with methionine is implemented with the DL-form (D'Mello and Lewis, 1978).

The methionine and cystine requirement, as g/kg dietary protein, of fingerling carp determined in Experiment 7 is similar to that found for channel catfish (Harding et al, 1977), but considerably lower than those reported for the rat, pig and chick (Mertz, 1969). Again, the estimated value in the present study is also lower than that found for the turkey (D'Mello, 1976).

As the fish used in Experiment 7 were much smaller than those used in Experiments 6 and 8 (Appendix B), the growth performance, in general, was less in the former experiment than in the latter. Hence, the intake of sulphur amino acid required to support maximum growth (Fig. 22) and protein deposition (Fig. 23) in Experiment 7 was much lower than that observed in Experiments 6 (Figs.20 and 21) and 8 (Figs. 24 and 25). The growth retardation and the lower deposition of carcass protein observed in the group given the highest dietary methionine level could be related to the toxic effect of the excessive level of methionine.

Further research is required as to whether carp are capable of utilising the α-keto and α-hydroxy acids of methionine.

c. The sparing action of cystine on methionine requirements

Of the sulphur-containing amino acids, only methionine is identified as an indispensable amino acid. This arises from the ability of animals to synthesise cystine from methionine. The reverse reaction, however, does not occur. Dietary cystine is therefore capable of replacing part, but not all, of the dietary needs for methionine.

Considerable work has been carried out with terrestrial vertebrates to determine the maximum amount of the animal's total sulphur amino acid requirements that can be satisfied by cystine. Studies with rats (Womack and Rose, 1941) have shown that the replacement value of cystine for methionine is 16.7%. More recently, Sowers et al (1972) have shown that cystine can contribute up to 64% of the total sulphur amino acid needs of the growing rat. Baker et al (1969) have shown that cystine can supply at least 56% of the total sulphur amino acid needs of young pigs. Several different estimates on the replacement value of cystine for methionine have been reported with chicks. Graber et al (1971b) have shown that dietary cystine can safely supply 55% of the sulphur amino acids required by these birds. This value is similar to that (54%) recently reported by Wheeler and Latshaw (1981), but slightly lower than that (52.7%) found by Sasse and Baker (1974).

A similar sparing action of cystine on methionine requirements was also observed with fish. Harding et al (1977) demonstrated that cystine could provide 60% of the dietary methionine

requirement of channel catfish. Jackson and Capper (1982) reported a similar value with tilapia, although this value was estimated by using only one concentration of dietary cystine. In the current investigation, the sparing action of cystine on the methionine requirement of carp was estimated to be at least 33%. Carp are therefore similar to endothermic animals and also to channel catfish and tilapia, in their ability to utilise a part of the cystine to satisfy their methionine requirements.

Experiments with chicks (Graber and Baker, 1971; Sasse and Baker, 1974) and rats (Stockland et al, 1973) have suggested that the dietary requirement for sulphur amino acids will be lower when a proper combination of methionine and cystine is fed, than when methionine alone is used to meet the total requirement. Similar observations were also reported with several species of fish (Halver et al, 1959; Cowey and Sargent, 1979; Kaushik and Luquet, 1980). For example, Halver et al (1959) have shown that chinook salmon were unable to sustain a maximum growth rate at a very low dietary cystine level (0.5 g/kg) and high methionine concentration (16.0 g/kg). However, chinook salmon exhibited a maximum growth rate when a better proportion of dietary methionine (5-6 g/kg) to cystine (10.0 g/kg) was ensured. Similar observations were also recorded with eel (Arai and Nose, unpublished data, cited in Cowey and Sargent, 1979).

In the present study it was not possible to formulate a basal diet low in methionine and completely deficient in cystine in order to determine the dietary methionine requirement of carp in the

absence of cystine. Nevertheless, the level of cystine adjusted in the basal diet was so low (1.1 g/kg) that it was not detected by the amino acid analyser. As shown earlier in Table 42, carp exhibited a maximum growth rate when the methionine and cystine concentrations were 7.8 and 3.3 g/kg diet respectively. On the other hand, carp failed to exhibit a maximum growth rate when the dietary levels of methionine and cystine were 11.0 and 1.1 g/kg respectively. These observations were more obvious when the growth rate (Fig. 26) and protein deposition (Fig. 27) data was plotted against the intake of sulphur amino acids. It may be concluded that carp are similar to chicks (Graber and Baker, 1971), rats (Stockland et al, 1973), chinook salmon (Halver et al, 1959), eel (Arai and Nose, unpublished data, in Cowey and Sargent, 1979) and rainbow trout (Kaushik and Luquet, 1980) in their requirements for a balanced combination of methionine and cystine in the diet.

In recent years attempts have been made to compare the responses of different animal species to graded intakes of the indispensable amino acids. The advantage of adopting such an approach in comparative studies has been previously demonstrated with several endothermic animals (D'Mello, 1976, 1978), and has already been speculated on with regard to various species of fish (Section IV-A-3). Similarities were seen in the arginine, valine, isoleucine and methionine plus cystine requirements of chicks and turkeys, and in the lysine requirements of carp, channel catfish and tilapia. In the case of methionine plus cystine, however, it has been suggested (D'Mello, 1978) that the variations in the dietary proportions of these amino acids and in the nature of

isomers used, may influence the position of the response curves when attempts are made to demonstrate the utilisation of sulphur amino acids by various animal species.

Fig. 38 shows the growth responses in relation to the intake of the sulphur amino acids in carp fed varying proportions of methionine and cystine at a water temperature of 25°C. In addition to the data obtained with carp fed identical diets in which the DL-isomer of methionine was used as a supplement and at temperatures of 20° (Experiment 6) and 25°C (Experiment 8), Fig. 38 also incorporates data obtained with carp offered similar diets but supplemented with the L- form of methionine, at 20°C. As can be seen from Fig. 38, the intake of sulphur amino acids required to promote a given growth rate in carp maintained under varying experimental conditions, is broadly similar. Ingestion of excessive amounts of methionine (Experiment 8) or cystine (Experiment 9) is responsible for the worse fit on the general response pattern. It appears that the utilisation of sulphur amino acids by carp is influenced by : (i) variation in the dietary proportions of methionine and cystine; (ii) the nature of isomers used; (iii) the imbalance of dietary cystine with respect to methionine. These findings, however, are in close agreement with the reports of D'Mello (1978), on the factors affecting the amino acid requirements of animals.

For the purposes of further comparison in the utilisation of the sulphur amino acid by various species of fish, the data presented in Fig. 38 was plotted in a further graph (Fig. 39). The

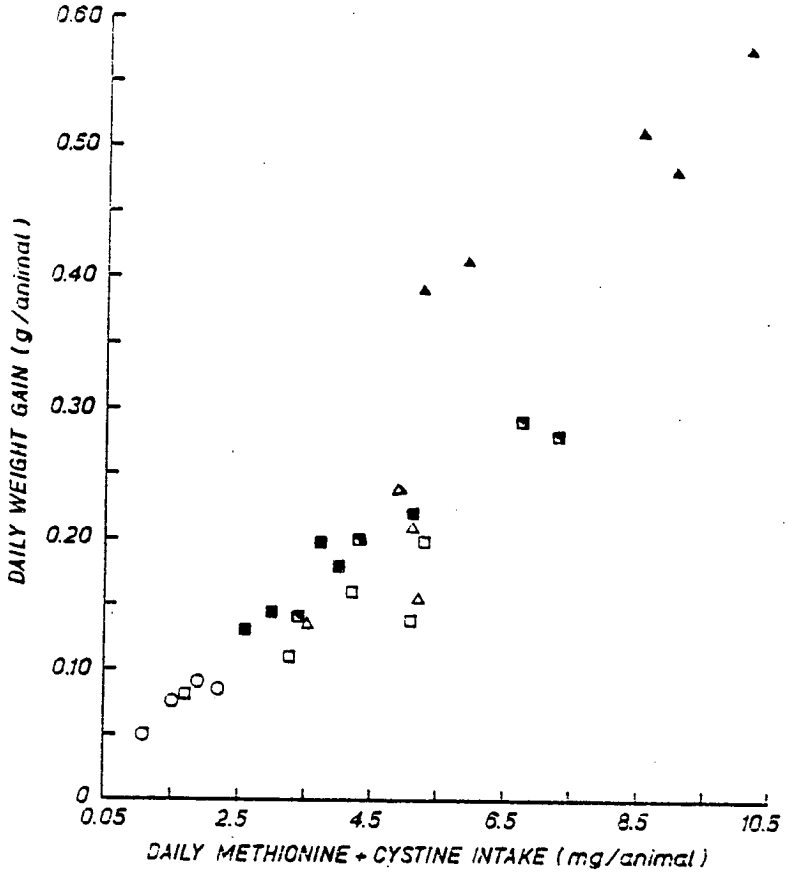


Fig. 38 Daily weight gain (g/fish) and daily methionine + cystine intake (mg/fish) of carp offered different concentrations of DL-methionine at 20 (◪) and 25°C (▲) and L-methionine at 20°C (□), and various proportions of methionine (○, ■, △) and cystine (in factorial combinations) at a water temperature of 25°C. Data from Experiments 6, 8, 7 and 9 respectively

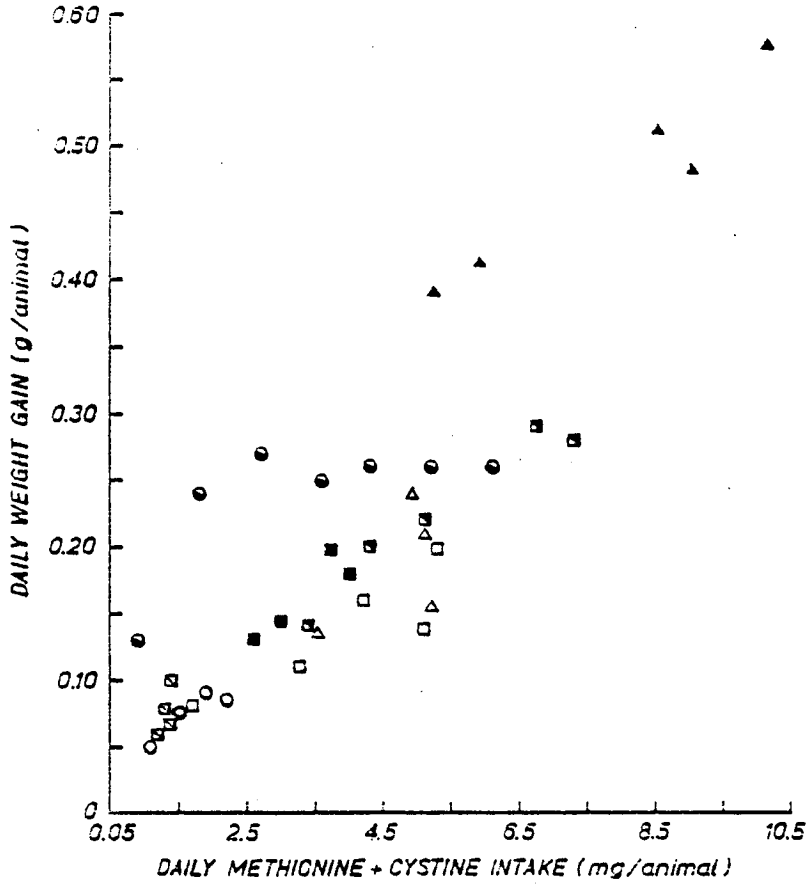


Fig. 39 Growth responses (g/fish/d) of tilapia, channel catfish and carp in relation to methionine + cystine intake (mg/fish/d). Tilapia data (▣, 25°C) from Jackson and Capper (1982); channel catfish data (◕, 20.7°C) from Harding et al (1977) and carp data (◪, 20°C; □, 20°C; ▲, 25°C and ●, ■, △, 25°C) from Experiments 6, 7, 8, and 9 respectively

responses of channel catfish (Harding et al, 1977) and tilapia (Jackson and Capper, 1982) to the intake of methionine and cystine can therefore be compared with those of carp in Fig. 39. As can be seen from the response patterns illustrated, the intake of sulphur amino acids required to promote a given growth rate remained broadly similar for carp maintained under the various experimental conditions and also for tilapia. The response data of Harding et al (1977) obtained with channel catfish, however, is not compatible with these results obtained in the case of carp and tilapia. The reason for this discrepancy could be mainly attributed to the fact that Harding and his colleagues used different methionine to cystine ratios, as compared with those used in the present study and by Jackson and Capper. The L-form of methionine and cystine used by Harding et al (1977) may have resulted in more efficient utilisation of the sulphur amino acids. Another explanation could be related to the different dietary concentrations of choline used by separate investigators. In the present study, for example, a dietary concentration of 9.0 g choline/kg was employed, whereas Harding et al (1977) used a dietary choline concentration of choline of 75 g/kg, and Jackson and Capper (1982) employed 4 g/kg diet in the case of tilapia. The exceptionally high concentration of choline incorporated into the diets by Harding et al (1977) could well explain the divergence in results obtained with channel catfish as compared with those for carp and tilapia.

Recent studies have shown that choline and betaine may affect the dietary methionine requirements of chicks. This effect arises from the function of these compounds as methyl group donors. Pesti

et al (1978) have shown that, as compared with methionine additions, cystine or sulphate supplements failed to promote growth or efficiency of food conversion in chicks fed diets deficient in methionine. In these diets, corn and soyabean meal were used as sources of protein. This failure was attributed to the lack of either the carbon skeleton of methionine or of available methyl groups. However, when the same diet was supplemented with choline or betaine, indistingushable responses were achieved as compared with those found in the case of methionine supplements. These observations indicate that the corn-soya diet was indeed lacking in methyl groups. From the results of Pesti et al (1978), it is clear that choline and betaine play a major role in influencing the methionine requirements (as g/kg diet) of chicks. Further work, however, is needed to investigate the effects of dietary choline and betaine on the methionine requirements, as dietary concentrations and daily intake, in chicks and other animals.

Another reason for discrepancies shown in the requirements of carp and tilapia with channel catfish for sulphur amino acids (Fig. 39) could relate to the various techniques used by different authors for assessing methionine and cystine in the diet, and to difficulties associated with determining these amino acids precisely (Section I-B-1). For example, in hydrolysis of protein with HCl and chromatography or ion-exchange resin columns, the methionine peak tends to disappear and is then replaced by a peak corresponding to methionine sulphoxide (Blackburn, 1978). The poor recovery of methionine sulphoxide prevents accurate methionine estimation. Cystine, on the other hand, is unstable during protein

or peptide hydrolysis (Blackburn, 1978). It does not actually undergo extensive degradation, but is converted to closely related derivatives which might be quantified by an automatic amino acid analyser (Blackburn, 1978).

From the foregoing discussion it becomes clear that more experiments should be conducted to determine the requirements of fish for sulphur amino acids. It is unlikely that fish species differ from endothermic terrestrial vertebrates in utilising sulphur amino acids, as clear similarities in the utilisation of methionine plus cystine by the latter animals have been discovered (D'Mello, 1976, 1978). The importance of a common methodology with exceptional attention to food intake, in determining the requirements of fish for sulphur amino acids, should also be emphasised.

In comparison with terrestrial vertebrates, data on methionine requirements of fish at different dietary levels of cystine is scarce. A clear relationship between dietary cystine levels and methionine requirements of various fish species cannot, therefore, be established.

Fig. 40 shows the relationships between dietary cystine levels and methionine requirements of rats, chicks, carp and channel catfish. As can be seen, methionine requirements of the rat and chick fall linearly with increasing dietary cystine concentrations up to a point represented by about 30 g cystine/kg dietary protein. After this point, methionine requirements become independent of

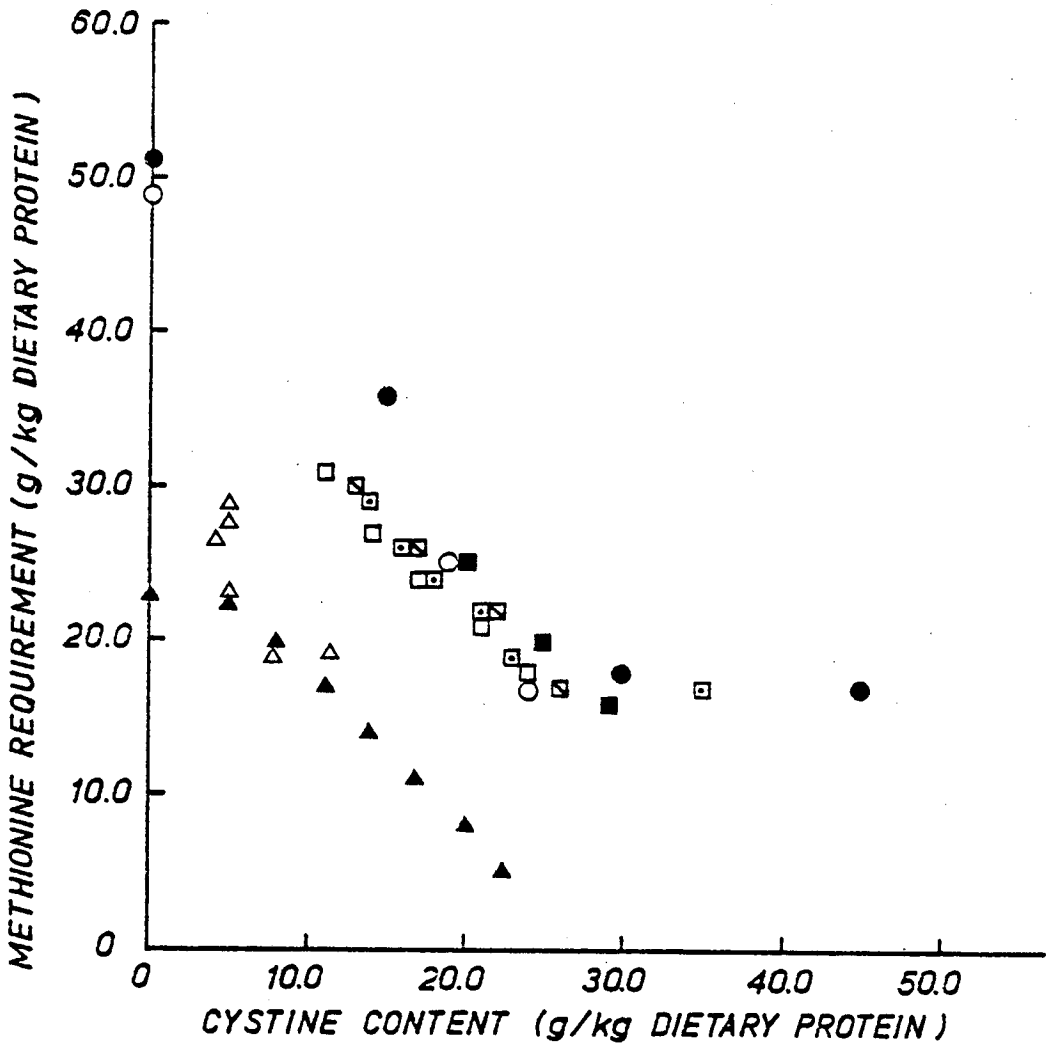


Fig. 40 Dietary methionine requirements (g/kg dietary protein) of rats, chicks, channel catfish and carp in relation to dietary cystine concentrations. Source of data: rats [(●), Sowers et al, 1972; (○), Stockland et al, 1973]; chick [(⊡), Wheeler and Latshaw, 1981; (□, ▨, ■), Graber et al, 1971a]; channel catfish [(▲), Harding et al, 1977] and carp [(△), Experiments 6, 7, 8 and 9]

dietary cystine concentrations. Carp showed the same pattern for the relationship between dietary cystine levels and methionine requirements as that demonstrated with rats and chicks. As shown in Fig. 40, the requirements of carp for methionine decreased linearly with increasing cystine levels up to a point represented by about 4 g cystine/kg dietary protein. However, although channel catfish also exhibited a linear fall in their methionine requirements with increasing dietary cystine concentrations, these requirements showed no signs of levelling off. The reason for this discrepancy, compared with patterns of other animals, may be due to the limited range of methionine and cystine concentrations used by Harding and his colleagues, and possibly to the fact that the replacement value of cystine for methionine was not investigated in factorial sequence. The replacement value of cystine for methionine, however, was found to vary depending upon species, age and criterion of measurement (Graber and Baker, 1971). It should therefore be recognised that differences in the replacement value of cystine for methionine may influence the position of the pattern of methionine to cystine conversion in carp (Experiment 9)and channel catfish (Harding et al, 1977) as compared with other animals. Whether the methionine requirements of other species of fish are dependent on dietary cystine concentration in the same manner as that observed with carp or terrestrial vertebrates requires further investigation.

d. Adverse effects of excess levels of sulphur amino acids

The adverse effects resulting from ingestion of diets

containing disproportionate amounts of amino acids have been well documented for several terrestrial animals (Harper, 1958; Sauberlich, 1961; Harper, 1964; Harper et al, 1970; D'Mello and Lewis, 1970a; 1970b; 1970c; Harter and Baker, 1978; Featherston and Rogler, 1978). Little is known, however, about the effect of ingestion of excessive amounts of amino acids on the growth performance of fish.

In the present study, it was found that ingestion of excess levels of the DL-form of methionine at a water temperature of 20°C did not produce an obvious adverse effect. At a water temperature of 25°C, the DL-form of methionine was found to be toxic. This result, however, could be related to the fact that fish maintained at the lower temperature consumed less methionine and cystine than those maintained at the higher temperature. These observations are also supported by the results (Figs. 26 and 27) obtained from Experiment 9, which was also conducted at 25°C.

The existence of antagonism of cystine on methionine utilisation has been reported with chicks fed suboptimal levels of dietary methionine (Featherston and Rogler, 1978). The growth depression observed with chicks as a result of ingestion of excessive amounts of cystine, was attributed to competition of the sulphur amino acids (of which methionine was already present in growth-limiting quantities) for a common site on the mucosal epithelial membrane of the intestine. However, Featherston and Rogler (1978) were not thoroughly convinced of this reason.

In Experiment 9, excessive intake of cystine was found to be toxic at the three levels of methionine investigated (Figs. 26 and 27). The biochemical reason for the adverse effect of excessive intake of cystine cannot be interpreted in the present studies. It should be recognised, however, that carp are similar to other terrestrial animals in their inability to tolerate excessive levels of sulphur amino acids.

Studies with rats (Sauberlich, 1961; Harter and Baker, 1978; Harper et al, 1970) have shown that methionine is the most toxic amino acid and that it produces the severest growth retardation when fed at excessive levels. A depressive effect of excess free methionine was also observed with rainbow trout (Kaushik and Luquet, 1980) and tilapia (Jackson and Capper, 1982). Whether the toxic effect of a dietary excess of methionine could be counteracted by increasing the intake of glycine and serine, as reported with rats (Benevenga and Harper, 1967), or by glycine and arginine, as demonstrated with chicks (D'Mello and Lewis, 1970a), needs to be investigated.

5. Tryptophan Requirements

The data obtained from Experiments 10 and 11 and presented in Tables 45 and 46 respectively, indicates that the dietary tryptophan requirement of fingerling carp (2.6 g/kg) is lower than the value (3.0g/kg) suggested by Nose (1978) for young carp. Again, the tryptophan requirement of carp, as determined in the present study, is higher than that (2.0 g/kg) found for chinook

salmon (Halver, 1965) and about twice that suggested for (1.2 g/kg) channel catfish (Wilson et al, 1978). The tryptophan requirement of eel (4.0 g/kg) reported by Arai and Nose (unpublished data, cited in NAS/NRC, 1981) is surprisingly higher than that of other fish species. In general, the tryptophan requirement, as g/kg dietary protein, of fish species is lower than that reported for endothermic animals (as shown earlier in Table 14). The lower tryptophan requirement of fish may relate to their inability to convert this amino acid to niacin.

Studies with terrestrial animals have shown that tryptophan requirements are considerably affected by the dietary concentration of niacin (Krehl et al, 1945; Almquist, 1959; Harper, 1964) since these animals are capable of converting tryptophan to niacin. Brook trout (Salvelinus fontinalis) (Poston and Dilorenzo, 1973), or salmonoids in general (Halver, 1970; Poston and Combs, 1980) and channel catfish (Wilson et al, 1978) were found to be unable to convert dietary tryptophan to niacin efficiently. Wilson et al (1978) suggested that the lower tryptophan requirement in fish species (Table 14) may be due to the inefficient conversion of tryptophan to niacin as indicated earlier. It may be concluded that carp, in contrast to endothermic animals, (Krehl et al, 1945; Almquist, 1959; Harper, 1959) resemble brook trout and channel catfish in their inability to convert dietary tryptophan to niacin efficiently.

The slightly poor growth performance of carp observed (Table 45) in Experiment 10, as compared to that achieved in experiment

11, was due to the particular (poultry) vitamin and mineral mixture used. However, the growth performance of fish obtained in the former experiment was sufficient to predict the tryptophan requirement of carp, although the growth rate was the only criterion used to assess this need. The daily intake of tryptophan required to promote maximum growth and carcass deposition of protein was found to be 1.7 mg/fish.

In both experiments, deficiency syndromes, such as scoliosis or lordosis, were not observed as they were in tests with chinook salmon (Halver, 1970), sockeye salmon (Halver and Shanks, 1960; Halver, 1970) and rainbow trout (Shanks et al, 1962; Halver, 1970). Fingerling carp are therefore similar to young carp (Nose, 1978) and tilapia (Mazid et al, 1978) in showing no scoliosis and lordosis symptoms when fed on diets deficient in tryptophan.

Tryptophan was found to be the second most toxic amino acid, after methionine, when fed in excessive levels to rats (Sauberlich, 1961). The growth retardation observed in Experiments 10 and 11 could be attributed to the excessive intake of tryptophan provided by the diets containing a high level (3.2 g/kg) of this amino acid. Whether this ill-effect can be reversed by adding other amino acids requires further investigation.

6. Histidine Requirements

The lack of a positive response to the varying concentrations of dietary histidine would indicate that within the range tested,

the histidine levels had no limiting effect. This result suggests a requirement of less than 5.2 g/kg diet. However, even this figure is much lower than that (8.0 g/kg diet) reported for carp fry (Nose, 1978) and eel (NAS/NRC, 1981). The histidine requirement of carp determined in the current investigation is also lower than that (7.0 g/kg diet) suggested for chinook salmon (Klein and Halver, 1970), but marginally higher than that (3.7 g/kg diet) estimated for channel catfish (Wilson et al, 1980). The requirement of carp for histidine (8.0 g/kg diet) reported by Nose (1978) could have been over-estimated. An examination of the data published by the latter investigator indicated that the daily specific growth rates of carp given diets containing 2.5, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 g histidine/kg were 0.9, 1.7, 1.9, 1.9, 2.1, 2.0 and 1.9 respectively. Indeed, there is a considerable difference in the growth rates of carp offered the diets containing 2.5 and 5.0 g histidine/kg. As can be seen from this data, the growth rates of carp, maintained on diets containing histidine levels over the range of 5.0 and 10.0 g/kg, did not differ greatly.

When expressed as a proportion of dietary protein, the histidine requirement of fingerling carp suggested in the present study (<14.97), is much lower than that of young carp (Nose, 1978), eel (NAS/NRC, 1981), chinook salmon (Klein and Halver, 1970) and channel catfish (Wilson et al, 1980). Again, when the estimated values, as g/kg dietary protein, from the present study are compared with those of other terrestrial animals, the requirement of carp for histidine is lower than that of the rat, pig and chick (Mertz, 1969), and the turkey (ARC, 1975).

The data presented in Tables 5 and 14 suggests that real differences exist between the various species of fish and terrestrial animals in their requirement, as dietary concentration, for histidine. Whether the reported variations in histidine requirements of different species of animal can be attributed to some genuine genetic differences in histidine utilisation or to differences in food intake needs to be investigated.

In the present study, however, it was found that the minimum daily intake of histidine required to support maximum growth was 1.6 mg/fish. This level of histidine intake was supplied from the basal diet which contained the lowest concentration of histidine.

The depression in growth rate and food intake observed with fish fed on the diet containing the highest level of histidine is similar to that reported by Sauberlich (1961) in the case of the rat. The growth retardation of this group was also accompanied by a marked reduction in carcass deposition of protein, fat and GE. Whether this adverse effect, caused by excessive levels of histidine intake, occurred as a result of an amino acid imbalance, needs to be investigated.

7. Threonine Requirements

The threonine requirement of carp could not be established as there were no growth responses to threonine levels of between 5.2 and 7.4 g/kg diet. However, the minimum daily intake of threonine required to support maximum growth and protein utilisation was

determined to be 3.2 mg/fish. This intake of threonine was provided by the basal diet. A highly significant reduction in carcass protein, and an equally significant increase in GE content, were found within the group given a dietary threonine concentration of 13.3 g/kg when these effects were compared with those of the initial sample. This result indicates that the basal diet was not limiting in threonine. From these findings the requirement of carp for threonine was estimated to be less than 8.41 g/kg diet (Table 52). This value is much lower than that (15.0 g/kg diet) found for young carp (Nose, 1978) and eel (NAS/NRC, 1981), but marginally lower than that suggested for chinook salmon (Halver et al, 1958). As the requirement of channel catfish (Wilson et al, 1978) for threonine is 5.3 g/kg diet, it is possible that the requirements of carp and channel catfish are of the same order.

When the requirement for threonine is expressed in terms of dietary protein, the estimated value (21.2 g/kg) for carp obtained from the present study is much lower than that reported for young carp (39.0 g/kg; Nose, 1978) and eel (36.0 g/kg; NAS/NRC, 1981), but marginally lower than those suggested for chinook salmon (22.0 g/kg; Halver et al, 1958) and channel catfish (22.1 g/kg; Wilson et al, 1978). Again, the estimated threonine requirement of carp, as g/kg dietary protein, is much lower than that determined for the rat, pig and chick (Mertz, 1969), and for the turkey (ARC, 1975). As shown earlier in Table 11, the requirement for dietary threonine differs from one species to another. In contrast the rat, pig, chick and turkey showed a relatively similar requirement for threonine as g/kg dietary protein (Table 14).

Whether various fish species and other terrestrial animals are similar in their utilisation of dietary threonine, needs to be established.

B. Problems involved in the experimental programme

The protein and amino acid requirements of carp were not investigated under all those conditions that influence the rate of voluntary food intake. However, the first experiment in the present study established the level of dietary protein required by fingerling carp, using intact protein sources. The requirements of carp for lysine and sulphur amino acids were examined in some detail since it is widely recognized that lysine and methionine are the first and second limiting amino acids in cereals. Due to the large number of experiments necessarily involved in this study, the requirements for arginine, isoleucine, leucine, phenylalanine and valine were not determined.

The choice of daily weight gain, as one of the main critera for assessing the requirements, was due to the ease of determination and to the high degree of accuracy inherent in a simple process of weighing. Data on amino acid intake, protein deposition and the efficiency of protein deposition were more informative, although this data relied entirely upon the degree of determination of the DM intake of fish in their aquatic habitat, and upon the results of the chemical analysis of the diets and/or fish tissue.

The accuracy and reliability of the above criteria would have

been enhanced if: (i) a higher number of fish (at least 30), having less variation in body weight (± 2 g) and size (± 1") had been used, (ii) more treatment replicates (3-4) had been employed, and (iii) more attention had been paid to the rejected diet by individual fish, to avoid further losses resulting from the schooling behaviour of the entire population. Although particular efforts were made to obtain fish of similar size and body weight, the variation in body weight was within the range of ± 8 g. The desirable fish were selected from the fish farm prior to transportation to the laboratory. Food losses from the rejected diets were minimised by immediate siphoning off of the rejected food. However, data on DM intake provided a fair degree of reliability to demonstrate the relationship between growth rate and protein utilisation in carp. All conclusions concerning amino acid requirements, as a dietary concentration or intake, of carp, were based entirely on the growth observations.

C. Future work on protein and amino acid requirements

As the dietary protein requirement of carp suggested in this thesis is similar to that reported for carp fry, it is imperative that further efforts be directed towards defining the stage of life at which the requirement could be dropped. Similar efforts should be directed towards estimating the protein requirement of fish under each of the various factors influencing food intake. The utilisation of protein in relation to protein intake under the different production facilities, also needs to be defined. These experiments must inevitably involve the measurement of food intake

and the use of more efficient facilities. The importance of employing semi-purified diets, particularly in the case of carp, has already been emphasised. Based on the findings of the present study, it is suggested that the values of each indispensable amino acid requirement determined by the above technique, would be more appropriate in the formulation of a practical diet for carp. Further data on the amino acid requirements of carp and other fish species would be of considerable interest for comparative purposes with terrestrial vertebrates.

The suggested criteria of observation are: daily growth rate, amino acid intake and efficiency of protein deposition. In particular, the aspects that warrant further detailed study are listed below: (i) The protein requirement of carp under different environmental conditions and at different stages of life, the ratio of total energy to protein energy, and the sparing action of carbohydrates on the protein requirement, still need to be defined. (ii) As dietary concentrations and daily intake, the requirements of carp for tryptophan, histidine and threonine need to be investigated under various experimental conditions. (iii) The adverse effects due to excessive intake of lysine, methionine (both DL- and L-form), tryptophan and histidine need to be examined in more detailed studies involving measurement of plasma amino acid concentrations. Whether this adverse effect as observed in the present study is reversible by the addition of other amino acids, needs detailed investigation with carp and other species of fish.

D. Concluding comments

The results of the present study indicate that the protein requirement of fingerling carp is similar to that of young carp. A level of 389 g protein/kg is therefore considered adequate for maximum growth and protein utilisation. Lower levels of dietary protein associated with high levels of non-protein energy (starch) may result in undesirable accumulation of fat in the carcass tissue of carp.

The requirements of carp for several indispensable amino acids have been defined in terms of dietary concentrations and daily intake. The growth responses obtained from diets formulated from intact protein sources and supplemented with synthetic amino acids, emphasise that carp are similar to tilapia, chinook salmon and channel catfish in utilising the free amino acids as dietary supplements.

The amino acid requirements of carp are compared with those of other species in terms of dietary concentrations (Table 55) and daily intake (Table 56). In general, results obtained from the present experimental programme suggest that the dietary requirements of carp for lysine, methionine or total sulphur amino acids, tryptophan, histidine and threonine are lower than those found by Nose (1978) using purified diets. The values given for carp based on the current study (Tables 55 and 56) may be regarded as the best available estimate of amino acid requirements for fingerling carp.

Food intake is influenced by a number of factors including environmental temperature. The effects of the water temperatures, 20 and 25°C, on the dietary lysine requirement and intake of carp have been demonstrated. This allowed a comparison to be made

TABLE - 56

Amino acid requirements, in terms of daily intake (mg/fish), of carp, channel catfish and tilapia.

	Carp fingerling		Channel catfish		Tilapia	
	growth rate g/fish/d	requirement mg/fish/d	growth rate g/fish/d	requirement mg/fish/d	growth rate g/fish/d	requirement mg/fish/d
Amino acid						
Histidine	0.11	1.6[1]	0.23	0.7[9]		
Lysine	0.26	6.0[2]	0.17	1.7[10]	0.08	1.6
	0.57	16.7[3]				
Sulphur amino acids	0.29	6.7[4]	0.24	0.6[11]	0.10	14.4
	0.18	4.2[5]				
	0.61	10.1[4]				
	0.20	3.0[6]				
	0.23	4.8[6]				
Threonine	0.12	3.0[7]	0.36	1.4[12]		
Tryptophan	0.19	1.7[8]	0.30	0.3[12]		

Source of data :

[1] Experiment 12
[2] Experiment 3 and 4
[3] Experiment 5
[4] Experiment 6 and 8
[5] Experiment 7
[6] Experiment 9
[7] Experiment 13
[8] Experiment 10 and 11
[9] Wilson et al (1980)
[10] Wilson et al (1977)
[11] Harding et al (1977)
[12] Wilson et al (1978)

Jackson and Capper (1982)

methionine required. An excessive intake of methionine and cystine, however, resulted in a growth depression. Similar ill-effects due to excess levels of lysine, tryptophan and histidine were also recorded.

Appendix A

TABLE - A1

Composition of the vitamin mixture used for all experiments. Concentrations are expressed in terms of contribution to the complete basal diet.

Vitamins	g/kg diet
Thiamin HCl	0.050
Riboflavin	0.200
Pyridoxine HCl	0.050
Choline bitartrate	9.024
Nicotinic acid	0.746
Ca-Pantothenate	0.497
Inositol	2.000
Biotin	0.005
Folic acid	0.015
Ascorbic acid	0.995
Cyanocobalamin	0.001
Menadione	0.040
α-Tocopherol acetate	0.398
	14.021
Cellulose filler	13.979
Total	28.000

[a]Caleb Brett (Grampian) Ltd., Aberdeen, Scotland
[b]except for Experiment 10.

TABLE - A2

Composition of the mineral mixture[a] used for all experiments[b]. Concentrations are expressed in terms of final contribution to the complete diet.

Minerals	Structure	g/kg diet	Ca	P	Mg	Fe	K	Cl	Al	Cu	Mn	Co	Zn	S	I	Na
Calcium biphosphate	$CaH_2(PO_4)2\ H_2O$	13.780	2.191	1.693	–	–	–	–	–	–	–	–	–	–	–	–
Calcium carbonate	$CaCO_3$	1.040	0.416	–	–	–	–	–	–	–	–	–	–	–	–	–
Magnesium carbonate	$MgCO_3$	1.820	–	–	0.525		–	–	–	–	–	–	–	–	–	–
Ferrous sulphate	$FeSO_4\ 7H_2O$	0.600	–	–	–	0.121	–	–	–	–	–	–	–	–	–	–
Potassium chloride	KCl	1.000	–	–	–	–	0.524	0.476	–	–	–	–	–	0.069	–	–
Aluminium sulphate	$Al_2(SO_4)3\ 16H_2O$	0.004	–	–	–	–	–	–	0.001	–	–	–	–	–	–	–
Copper sulphate	$CuSO_4\ 5H_2O$	0.020	–	–	–	–	–	–	–	0.005	–	–	–	0.001	–	–
Manganous sulphate	$MnSO_4\ 4H_2O$	0.072	–	–	–	–	–	–	–	–	0.026	–	–	0.003	–	–
Potassium iodide	KI	0.004	–	–	–	–	0.001	–	–	–	–	–	–	0.015	–	–
Cobalt sulphate	$CoSO_4\ 7H_2O$	0.020	–	–	–	–	–	–	–	–	–	0.004	–	–	0.003	–
Sodium chloride	NaCl	1.600	–	–	–	–	–	0.970	–	–	–	–	–	0.002	–	–
Zinc sulphate	$ZnSO_4\ 7H_2O$	0.080	–	–	–	–	–	–	–	–	–	–	0.032	0.016	–	0.629
Total		20.00	2.607	1.693	0.525	0.121	0.525	1.446	0.001	0.005	0.026	0.004	0.032	0.106	0.003	0.629

[a] Caleb Brett (Grampian) Ltd, Aberdeen, Scotland
[b] except experiment 10

TABLE - A3

Composition of the mineral and vitamin mixture[a,b], expressed in terms of the final concentration contributed to 1 kg of the diet.

Vitamins, etc.		Minerals	
Retinol	3.99 mg	Ca	13860.00 mg
Cholecalciferol	1.00 mg	P	3320.00 mg
∝-tocopherol acetate	4.43 mg	Na	1330.00 mg
Menadione sodium bisulphite	1.10 mg	Cl	2000.00 mg
Riboflavin	4.43 mg	Mn	77.60 mg
Folic acid	2.22 mg	Zn	55.43 mg
Cyanocobalamin	0.01 mg	Fe	22.17 mg
Nicotinic acid	22.17 mg	Cu	2.22 mg
Calcium pantothenate	11.09 mg	I	1.11 mg
Choline chloride	775.98 mg	Co	0.11 mg
Virginiamycin (growth stimulant)	5.54 mg	Se	0.11 mg
BHT (antioxidant)	140.00 mg	Mo	2.22 mg
Pancoxin (coccidiostat)	140.00 mg		

[a] used only in experiment 10
[b] D'Mello (1973)

Appendix B

TABLE - B1

Experiment 1 - Chemical composition of diets containing 6 levels of dietary protein.

Diet	Dietary protein level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
1P1	180.6	909.8	180.6	81.3	39.5	17.0
1P2	243.2	913.9	243.3	82.5	45.0	17.4
1P3	281.7	914.7	281.7	84.7	48.7	17.5
1P4	334.4	916.1	334.4	84.9	54.2	18.1
1P5	389.3	916.1	389.3	88.3	58.6	18.2

TABLE - B2

Experiment 2 - Chemical composition of diets containing 2 protein levels and 3 lysine concentrations in factorial combinations.

Diet	Dietary protein level g/kg DM	Dietary lysine level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
2P1L0	216.4	11.8	913.5	216.4	84.7	53.1	17.5
2P1L1	222.1	13.9	914.0	222.1	83.6	51.3	17.8
2P1L2	215.6	16.1	915.4	215.8	83.5	49.7	17.5
2P2L0	307.3	12.6	917.9	307.3	80.7	81.0	17.3
2P2L1	294.8	14.8	916.2	294.8	81.6	69.3	17.6
2P2L2	297.1	16.6	936.8	297.1	79.8	63.5	18.0

TABLE - B3

Experiments 3, 4 and 5 - Chemical composition of diets containing different levels of lysine.

Diet	Dietary lysine level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
Experiment 3:						
3L0	12.1	953.2	450.6	-	60.8	18.8
3L1	14.2	952.3	445.8	-	61.4	18.8
3L2	16.3	952.2	446.6	-	64.6	18.7
3L3	18.5	949.7	443.7	-	62.2	18.8
3L4	20.6	948.2	463.6	-	61.6	18.8
3L5	22.7	947.6	465.2	-	61.3	18.8
Experiment 4:						
4L0	12.5	921.3	429.3	83.8	64.4	18.5
4L1	14.5	929.7	447.1	84.4	63.4	18.4
4L2	16.7	929.8	444.2	82.1	61.8	18.5
4L3	18.8	931.0	438.6	81.3	62.5	18.4
Experiment 5:						
5L0	12.4	928.0	408.3	83.7	56.7	19.1
5L1	14.6	928.6	397.4	82.6	55.6	18.9
5L2	16.7	928.4	405.0	83.7	55.6	19.1
5L3	18.9	928.9	404.3	81.8	56.1	18.8
5L4	21.0	929.0	404.9	81.8	56.4	18.8
5L5	23.2	929.4	407.3	82.2	57.4	18.8

TABLE - B4

Experiments 6, 7 and 8 - Chemical composition of diets containing graded levels of methionine.

Diet	Dietary methionine level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
Experiment 6:						
6M0	6.1	919.3	388.5	72.4	28.3	18.2
6M1	7.7	919.4	408.8	72.4	30.2	18.3
6M2	9.4	919.1	400.3	72.4	29.0	18.4
6M3	11.0	918.7	381.0	72.8	28.6	18.0
6M4	12.6	919.0	386.1	72.4	29.8	18.0
Experiment 7:						
7M0	6.1	926.2	373.8	71.5	30.5	18.5
7M1	7.6	936.0	412.6	72.0	38.0	18.3
7M2	9.3	924.7	411.2	73.9	27.4	18.2
7M3	10.9	926.1	420.1	72.6	30.0	18.2
7M4	12.5	926.4	386.3	72.6	29.1	18.5
Experiment 8:						
8M0	6.1	922.5	384.4	71.8	28.8	18.8
8M1	7.7	922.0	387.4	72.2	19.8	18.4
8M2	9.3	922.0	386.9	72.2	28.2	18.3
8M3	11.0	920.2	387.3	72.3	28.1	18.7
8M4	12.6	921.0	393.0	71.5	27.4	18.5

TABLE - B5

Experiment 9 - Chemical composition of diets containing graded levels of methionine and cystine in factorial combination.

Diet	Dietary methionine level g/kg DM	Dietary cystine level g/kg DM	DM g/kg Diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
9M0C0	4.5	1.1	919.7	416.2	72.0	27.8	18.3
9M0C1	4.5	2.2	921.0	426.1	73.0	28.2	18.2
9M0C2	4.5	3.3	922.9	419.7	72.1	28.7	18.1
9M0C3	4.5	4.3	922.1	426.3	72.9	26.9	18.1
9M1C0	7.8	1.1	917.9	446.6	71.0	28.2	18.2
9M1C1	7.8	2.2	919.3	447.4	72.4	29.2	18.1
9M1C2	7.8	3.3	919.6	412.6	73.1	30.8	17.9
9M1C3	7.8	4.3	919.3	384.6	73.1	30.4	17.8
9M2C0	11.0	1.1	918.6	451.6	73.5	31.6	18.2
9M2C1	11.0	2.2	919.3	413.4	73.5	33.0	18.1
9M2C2	11.0	3.3	920.0	396.3	71.6	30.6	18.1
9M2C3	11.0	4.3	920.6	400.6	70.4	33.2	17.9

TABLE - B6

Experiments 10 and 11 - Chemical composition of experimental diets containing graded levels of dietary tryptophan.

Diet	Dietary tryptophan level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
Experiment 10:						
10T0	1.5	919.9	409.6	75.0	48.6	17.9
10T1	2.1	922.0	419.8	74.8	48.2	17.8
10T2	2.6	923.3	429.0	74.7	47.6	17.9
10T3	3.2	920.6	417.7	75.3	48.7	18.1
Experiment 11:						
11T0	1.5	917.3	411.1	73.3	28.9	18.3
11T1	2.1	916.1	421.1	73.7	29.3	18.1
11T2	2.6	916.4	393.1	73.3	28.8	18.3
11T3	3.2	915.2	397.7	72.7	29.4	18.4

TABLE - B7

Experiments 12 and 13 - Chemical composition of diets containing graded levels of dietary histidine (Experiment 12) or threonine (Experiment 13).

Diet	Dietary histidine level g/kg DM	DM g/kg diet	Protein g/kg DM	Fat g/kg DM	Ash g/kg DM	GE MJ/kg DM
Experiment 12:						
12H0	5.2	926.1	347.4	76.0	43.4	17.7
12H1	5.7	927.3	384.1	74.8	41.6	18.1
12H2	6.3	928.1	389.7	75.5	41.3	17.7
12H3	6.8	926.5	396.8	64.8	41.0	17.9
12H4	7.4	927.1	391.6	75.6	40.3	17.8
Experiment 13:	Dietary threonine level g/kg DM					
13Th0	8.4	921.7	382.5	74.1	25.4	18.0
13Th1	10.0	922.1	381.3	74.4	26.2	18.0
13Th2	11.7	922.7	395.8	71.0	25.1	18.0
13Th3	13.3	922.1	398.2	75.9	25.0	18.1
13Th4	15.0	919.7	390.0	73.8	25.6	18.1

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